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GENETICS

A Periodical Record of Investigations Bearing on Heredity and Variations

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C. Nägeli

FURTHER GENETICAL STUDIES OF *APLOCHEILUS LATIPES*

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Received February 25, 1929

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INTRODUCTION

The genetic studies on a fresh-water fish, *Aplocheilus latipes*, carried on up to date since 1921, when my earlier investigations were reported, have revealed some new and interesting facts. The present paper is a report upon them. As it is a continuation of the former paper, I will here summarize some of the former results which are necessary to an understanding of the present experiments.

Among the color varieties of *Aplocheilus latipes*, orange red (briefly red) and white are sex-linked. Red (*R*) is dominant to white (*r*) and both genes are located in the sex chromosomes, X or Y. The sex inheritance is of the Drosophila type, the female being homogametic and the male heterogametic. The homozygous red male possesses the dominant gene *R* in both the X and the Y chromosomes. The cross of white female by red male produces heterozygous red offspring of both sexes, females **X*R*X*r*** and males **X*r*Y*R***. When the heterozygous red male **X*r*Y*R*** is mated to white females **X*r*X*r***, all male offspring are red and the females white. Here the sex linkage of red and white is well manifested. As the epigenetic gene *R* is located in the Y chromosome, it is inherited only in the masculine line, and the male is always red and the female white; and this strain may be perpetuated by mating the offspring *inter se*. Sometimes crossing over between X and Y chromosomes takes place, and the gene *R* is transferred from Y to X and there result red females (**X*R*X*r***) and white males (**X*r*Y*r***).

A white male **X*r*Y*r*** mated with a heterozygous red female **X*R*X*r*** yields red and white offspring in both sexes and in equal proportions (**X*R*X*r***,

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X*r***X***r*, **XR**Y*r*, **X***r*Y*r*). Among these offspring the red male possesses the *R* gene not in a Y but in an X chromosome, in contrast to the original red male.

To determine the frequencies of the transfer of the *R* gene from the X to the Y chromosome, and conversely, from the Y to the X, was the principal aim of the present experiments. For this purpose the two kinds of heterozygotic red male, **X***r*Y*R* and **XR**Y*r*, were repeatedly crossed with white females, **X***r***X***r*, and the exceptional offspring which resulted from crossing over were counted. If the two chromosomes, X and Y, were homologous, and crossing over took place between them with regularity, the frequency of transfer of the *R* gene from Y to X and from X to Y would be equal. A remarkable difference, however, was found between them. Moreover, a striking exceptional red male was found among the offspring of an **XR**Y*r* male, and breeding tests showed that it must have the genetical formula XXY.

CROSSING OVER BETWEEN THE X AND THE Y CHROMOSOMES

(a) *Transfer of the R gene from* Y *to* X.

Heterozygous red males (**X***r*Y*R*) were repeatedly mass-crossed to white females (**X***r***X***r*) and the production was noted of exceptional red females (**XR**X*r*) and white males (**X***r*Y*r*). These exceptional forms result from crossing over,—in this case, transfer of the *R* gene from the Y to the X chromosome. Their numbers are given in table 1.

TABLE 1

Red males (**X***r*Y*R*)×*White females* (**X***r***X***r*).

YEAR OF BREEDING	NORMAL OFFSPRING		EXCEPTIONAL OFFSPRING	
	WHITE FEMALE, X*r*X*r*	RED MALE, X*r*Y*r*	RED FEMALE, X*r*X*r*	WHITE MALE, X*r*Y*r*
1919	156	148	3	0
1920	147	154	0	3
1922	754	748	1	8
1923	349	373	0	0
1924	724	708	1	1
1925	1817	1945	4	7
	3947	4076	9	19
	8023		28	

The ratio of the exceptional to normal offspring is about 1:300

(b) *Transfer of the R gene from* X *to* Y.

The heterozygous red males (**XR**Y*r*) which have the *R* gene in an X

chromosome, and which are the offspring of a heterozygous red female mated to a white male, were mass-crossed to white females **X*r*X*r***. The normal offspring were red females (**X*R*X*r***) and white males (**X*r*Y*r***), but some exceptional white females and red males were obtained. The results are summarized in table 2.

TABLE 2

Red male (**X*R*Y*r***) × *White female* (**X*r* X*r***).

YEAR OF BREEDING	NORMAL OFFSPRING		EXCEPTIONAL OFFSPRING	
	RED FEMALE, X*r*X*r*	WHITE MALE, X*r*Y*r*	WHITE FEMALE	RED MALE
1921	484	450	2	0
1922	511	465	0	2 (A, B)
1923	574	574	2	4 (C, D, E, F)
1924	994	975	1	3 (G, H, J)
	2563	2437	5	9
	5000		14	

The ratio of the exceptional to normal offspring is about 1:360. It is very near to that in table 1, so that, at first sight, one might be induced to conceive all of these exceptional forms as the result of crossing over between X and Y chromosomes. The breeding experiments, however, showed that two males, A and B, from the 1922 breeding, and two males, C and D, from the 1923 breeding, and all three males, G, H, and J from the 1924 breeding had an unexpected genetic constitution resulting from non-disjunction of X, and being in formula **X*R*X*r*Y*r***, and not **X*r*Y*R***, as expected, as will be shown later in this paper.

The two remaining males, E and F, were true crossover forms and when mated to white females produced white females and red males in equal proportions. The offspring of E were 22 white females and 22 red males; the offspring of F were 83 white females and 86 red males.

As to the exceptional white females, I was unable to obtain an appropriate mate to determine whether they had resulted from crossing over, and so had the genetic formula **X*r*X*r***, or from non-disjunction, and so had the genetic formula **X*r*O**. Consequently, in calculating the frequency of crossing over, it will be safe to neglect the exceptional females and to take into account the males only.

The total sum of the males is 2446, of which two (E, F) are crossovers, the ratio being about 1200:1. The difference between this ratio and that mentioned in table 1 (1:360) is too great to be passed by with-

out any consideration. The *R* gene was found to have removed from the Y chromosome to the X about four times oftener than from X to Y, or, in other words, the Y chromosome has lost the *R* gene much easier than has the X. A similar difference was found by WINGE (1923). A gene, elongatus (e_l), which produces the elongation of the caudal fin in Lebistes, removed from the Y chromosome to the X four times out of 68 and from the X chromosome to the Y only once in 73 cases. Nevertheless, WINGE (1923, 1927) has concluded that the X and Y chromosomes are essentially homologous, and that the only difference between them consists in the location of an epistatic male-determining gene in the Y chromosome. He did not lay much stress upon this difference, though he indicated it as remarkable.

If the crossing over between the X and Y chromosomes takes place simply by chance and no other cause interferes with it, the frequencies of the transfer of the *R* gene in both directions should be equal, and no preponderance on either side should take place. At present I am unable to elucidate the cause of this difference in the direction of crossing over. Whether it is effected by a dissimilarity in structure of the X and Y chromosomes or by the action of some gene or genes situated in one of them is not known. However, the tendency of the Y chromosome more frequently to lose the dominant gene and less often to regain it is well established in our fish and also in Lebistes; and if this difference may be supposed to occur also in other animals, we have here, perhaps, a plausible explanation of the origin of the so-called empty Y chromosome of Drosophila where crossing over must have taken place so repeatedly between the X and Y chromosomes that the Y chromosome has gradually lost its dominant genes, eventually to become quite empty.

NON-DISJUNCTIONAL MALES

The exceptional red males A, B, C, D, G, H, and J of table 2, which showed no peculiarity in structure, either macroscopic or microscopic, of their genital glands, when crossed with the white females, produced the remarkable results shown in table 3. The progeny were mainly females, red and white in equal proportions, while the males were few in number or none at all.

The total results are 996 red and 953 white females and 11 red and 8 white males. Were the genetic constitution of the exceptional males ***XrYR***, which is to be expected if they were produced by the crossing over of the *R* gene from the X to the Y chromosome, their progeny should be white females and red males in equal numbers. As the experimental re-

TABLE 3

Primary non-disjunctional red male×white females (in all crosses 3 females were mated to a single male).

NUMBER AND YEAR OF BREEDING	MALE PARENT	OFFSPRING				RATIO
	RED ♂	RED ♀	WHITE ♀	RED ♂	WHITE ♂	
3 B. 1923	A	33	32	0	0	
4 B. 1923	B	55	36	2	1	30:1
(These three male offspring, two red and one white, were lost by an unfortunate accident before they were genetically studied).						
3 B. 1924	C	20	26	0	1 (K)	50:1
2 B. 1925	C	265	271	0	0	
4 B. 1924	D	9	25	0	0	
3 B. 1925	D	255	260	1 (L)	1 (M)	250:1
4 B. 1925	G	78	64	0	0	
5 B. 1925	H	125	91	1(N)	0	200:1
6 B. 1925	J	156	148	7 (P_1-P_7)	5(P_8-P_{12})	25:1
	Total	♀1949		♂19		100:1

sults do not correspond to this expectation, they are evidently not the product of crossing over, and their genetic constitution should be different from this expectation. The production of the red and white females makes it necessary to consider that the male parent had two X's, an **X***R* and an **X***r*. The genetic formula having a double dose of X is characteristic of females, but as these individuals are male, not female, they must necessarily have some male-determining factor which is more powerful than two X's. The phenomenon may be explained fairly well by the supposition that all these exceptional red males are the result of non-disjunction between sex chromosomes and have a Y chromosome in addition to two X's. The presence of a Y chromosome in these males will be fully substantiated when they, or their male progeny, produce the normal male of the constitution XY, which yields males and females in equal proportions. Such males were obtained, actually, among their offspring. Such, for example, were the red male N derived from male H, reared in breeding 5 in 1925 (table 3), and the white male $K_{2,1}$, a great-grandson of male C, reared in breeding 2 in 1926. (See table 4).

WINGE (previously cited), investigating crossing over between the X and the Y chromosomes in Lebistes, concluded that the Y chromosome carries a dominant male-determining gene. His conclusion is based on the supposed homology between the X and the Y chromosomes, inferred from

TABLE 4, PART 1

Offspring of secondary non-disjunctional males (In all crosses 3 females were mated to a single male).

NUMBER AND YEAR OF BREEDING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀	♀	♂	♂	♀	♂	
4 B. 1925	K white (3B. 1924)	Sister white		129 white		10 (K_1–K_{10}) white	129	10	10:1
11 B. 1926	L red (3B. 1925)	White	461 red	383 white	0	0	844	0	
12 B. 1926	M white (3B. 1925)	Wild brown	232 brown		1 (M_1) brown		232	1	200:1
13 B. 1926	M white (3B. 1925)	Sister red	113 red	108 white	1 (M_2) red		221	1	
14 B. 1926	N red (5B. 1925)	Wild brown	133 brown		156 brown		133	156	1:1
15 B. 1926	P_1 red (6B. 1925)	Red	190 red	68 white	0	0	258	0	
16 B. 1926	P_2 red (6B. 1925)	White	281 red	268 white	0	1 ($P_{2,1}$) white	549	1	500:1
17 B. 1926	P_3 red (6B. 1925)	Red	237 red	83 white	3 ($P_{3,1}$–$P_{3,3}$)* red	1 ($P_{3,4}$) white	320	4	80:1
18 B. 1926	P_4 red (6B. 1925)	Wild brown	248 brown		0		248	0	
19 B. 1926	P_5 red (6B. 1925)	Wild brown	303 brown		0		303	0	
20 B. 1926	P_6 red (6B. 1925)	White	130 red	138 white	1 ($P_{6,1}$) red	4 ($P_{6,2}$–$P_{6,5}$) white	268	5	50:1
21 B. 1926	P_7 red (6B. 1925)	White	246 red	238 white	0	0	484	0	
22 B. 1926	P_8 white (6B. 1925)	White		257 white		1 ($P_{8,1}$) white	257	1	250:1
23 B. 1926	P_9 white (6B. 1925)	Red	250 red	240 white	0	1 ($P_{9,1}$) white	490	1	500:1
24 B. 1926	P_{10} white (6B. 1925)	Wild brown	343 brown		0		343	0	
25 B. 1926	P_{11} white (6B. 1925)	White		380 white		1 ($P_{11,1}$) white	380	1	400:1
26 B. 1926	P_{12} white (6B. 1925)	Red	98 red	86 white	0	0	184	0	
			Total, exclusive of the offspring of N				5510	25	

* $P_{3,2}$ was an abnormal male and yielded no offspring.

TABLE 4, PART 2

NUMBER AND YEAR OF BREEDING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀	♀	♂	♂	♀	♂	
1 B. 1926	K_1 white (4B. 1925)	Red	189 red	173 white	0	1 ($K_{1,1}$) white	362	1	350:1
2 B. 1926	K_2 white (4B. 1925)	Sister white		264 white		4 ($K_{2,1}$—$K_{2,4}$) white	264	4	70:1
3 B. 1926	K_3 white (4B. 1925)	Sister white		200 white		4 ($K_{3,1}$—$K_{3,4}$) white	200	4	50:1
4 B. 1926	K_4 white (4B. 1925)	Wild brown	346 brown		0		346	0	
5 B. 1926	K_5 white (4B. 1925)	White		280 white		0	280	0	
6 B. 1926	K_6 white (4B. 1925)	Wh		346 white		4 ($K_{6,1}$—$K_{6,4}$) white	346	4	90:1
7 B. 1926	K_7 white (4B. 1925)	Red	117 red	124 white	1 ($K_{7,1}$) red	0	241	1	240:1
8 B. 1926	K_8 white (4B. 1925)	White		142 white		0	142	0	
9 B. 1926	K_9 white (4B. 1925)	Red	177 red	189 white	0	1 ($K_{9,1}$) white	366	1	360:1
10 B. 1926	K_{10} white (4B. 1925)	White		360 white		8 ($K_{10,1}$—$K_{10,8}$) white	360	8	40:1
24 B. 1927	M_1 brown (12B. 1926)	White	59 brown 38 red	50 blue 24 white	0	0	171	0	
25 B. 1927	M_2 red (13B. 1926)	Red	127 red	58 white	13 red	6 white	185	19	10:1
26 B. 1927	$P_{2,1}$ white (16B. 1926)	White variegated		55 white variegated 40 white		0	95	0	

TABLE 4, PART 2 (*continued*)

NUMBER AND YEAR OF BREEDING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀	♀	♂	♂	♀	♂	
27 B. 1927	$P_{2,1}$ red (17 B. 1926)	White	24 red	26 white	0	0	50	0	
28 B. 1927	$P_{2,3}$ red (17B. 1926)	White	25 red	15 white	0	0	40	0	
29 B. 1927	$P_{3,4}$ white (17B. 1926)	Red	87 red	68 white	2 red	1 white	155	3	50:1
30 B. 1927	$P_{6,1}$ red (20B. 1926)	White	74 red	53 white	0	0	127	0	
31 B. 1927	$P_{6,2}$ white (20B. 1926)	Red	22 red	23 white	1 red	0	45	1	50:1
32 B. 1927	$P_{6,3}$ white (20B. 1926)	Red	61 red	42 white	0	1 white	103	1	100:1
33 B. 1927	$P_{6,4}$ white (20B. 1926)	Red	47 red	47 white	4 red	1 white	94	5	20:1
34 B. 1927	$P_{6,5}$ white (20B. 1926)	Red	58 red	60 white	2 red	7 white	118	9	10:1
35 B. 1927	$P_{8,1}$ white (22B. 1926)	Red	49 red	31 white	0	0	80	0	
36 B. 1927	$P_{9,1}$ white (23B. 1926)	Red	65 red	69 white	8 red	11 white	134	19	7:1
37 B. 1927	$P_{11,1}$ white (25B. 1926)	Brown	49 brown 22 red	33 blue 23 white	0	1 blue	127	1	120:1
						Total	4431	81	

TABLE 4, PART 3

NUMBER AND YEAR OF BREEDING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀	♀	♂	♂	♀	♂	
1 B. 1927	$K_{1,1}$ white (1B. 1926)	Sister red	81 red	78 white	1 red	1 white	159	2	80:1
2 B. 1927	$K_{2,1}$ white (2B. 1926)	White variegated		75 white variegated 31 white		79 white variegated 32 white	106	111	1:1
3 B. 1927	$K_{2,2}$ white (2B. 1926)	White variegated		34 white variegated 23 white		17 white variegated 10 white	57	27	2:1
4 B. 1927	$K_{2,3}$ white (2B. 1926)	Brown	71 brown 50 red	51 blue 52 white	2 brown	1 blue	224	3	70:1
5 B. 1927	$K_{2,4}$ white (2B. 1926)	Red	90 red	66 white	17 red	11 white	156	28	6:1
6 B. 1927	$K_{3,1}$ white (3B. 1926)	Brown	30 brown 38 red	31 blue 33 white	2 red	1 white	132	3	40:1
7 B. 1927	$K_{3,2}$ white (3B. 1926)	Brown	52 brown 55 red	37 blue 31 white	3 red	1 blue	175	4	40:1
8 B. 1927	$K_{3,3}$ white (3B. 1926)	Brown	27 brown 13 red	24 blue 16 white	0	0	80	0	
9 B. 1927	$K_{3,4}$ white (3B. 1926)	Brown	20 brown 14 red	18 blue 6 white	0	1 blue	58	1	60:1
10 B. 1927	$K_{6,1}$ white (6B. 1926)	White variegated		2 white variegated 2 white		0	4	0	
11 B. 1927	$K_{6,2}$ white (6B. 1926)	Red	75 red	76 white	10 red	4 white	151	14	10:1
12 B. 1927	$K_{6,3}$ white (6B. 1926)	Red	70 red	59 white	4 red	1 white	129	5	30:1
13 B. 1927	$K_{6,4}$ white (6B. 1926)	Red	47 red	47 white	7 red	7 white	94	14	7:1
14 B. 1927	$K_{7,1}$ red (7B. 1926)	White	49 red	34 white	4 red	8 white	83	12	7:1

TABLE 4, PART 3 (*continued*)

NUMBER AND YEAR OF BREEDING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀	♀	♂	♂	♀	♂	
15 B. 1927	$K_{9,1}$ white (9B. 1926)	Red	71 red	72 white	5 red	13 white	143	18	8:1
16 B. 1927	$K_{10,1}$ white (10B. 1926)	Brown	61 brown 29 red	64 blue 21 white	1 brown	0	175	1	170:1
17 B. 1927	$K_{10,2}$ white (10B. 1926)	Brown	91 brown 47 red	65 blue 40 white	1 brown 1 red	2 blue 1 white	243	5	50:1
18 B. 1927	$K_{10,3}$ white (10 B. 1926)	Brown	90 brown 68 red	86 blue 57 white	2 brown 2 red	1 blue 2 white	301	7	40:1
19 B. 1927	$K_{10,4}$ white (10 B. 1926)	Brown	60 brown 44 red	53 blue 25 white	2 red	2 blue 1 white	182	5	40:1
20 B. 1927	$K_{10,5}$ white (10B. 1926)	Brown	64 brown 38 red	51 blue 39 white	0	1 white	192	1	200:1
21 B. 1927	$K_{10,6}$white (10B. 1926)	Red	139 red	141 white	4 red	2 white	280	6	50:1
22 B. 1927	$K_{10,7}$white (10B. 1926)	Red	139 red	131 white	7 red	6 white	270	13	20:1
23 B. 1927	$K_{10,8}$ white (10B. 1926)	Red	79 red	72 white	23 red	20 white	151	43	4:1
Total, exclusive of the offspring of $K_{2,1}$ and $K_{2,2}$							3382	185	

the occurrence of crossing over between them. But, as already described, the crossing over in our fish and also in Lebistes does not occur with equal frequency in both directions so that the homology of an X to a Y chromosome is questionable. The existence of a dominant male-determining gene or genes in the Y chromosome, however, is fully established

in the present case, where the non-disjunctional forms XXY are found to be males.

All the seven exceptional red males above mentioned were primarily non-disjunctional. They were products of the mass-breeding of heterozygous red males (**X*R*Y*r***) with white females (**X*r*X*r***). A question may be raised as to whether some of their parents might not have been previously non-disjunctional, and they themselves secondary forms. But when we examine the pedigree of the parents there will be no room for doubt. We will consider the male side first. If some of the parental red males were non-disjunctional, their mating to white females should have produced white females in abundance, just as happened in the mating of the primary non-disjunctional males of table 3. But this did not happen here, for the white females were very few, even less then the non-disjunctional males (table 2). Second, the possibility that some white female parents might have been non-disjunctional and of the formula **X*r*X*r*X*r*** is also negligible when the genetic constitution of their progenitors is investigated. They were the progeny of heterozygous red females **X*R*X*r*** and white males **X*r*Y*r***, which produced red and white females and males in equal proportions. Any non-disjunction among **X*R*X*r*** and **X*r*Y*r*** is unable to produce **X*r*X*r*X*r*** forms.

Now, inasmuch as these seven non-disjunctional males were red, it is clear that the non-disjunction took place in the male parent. Consequently, an **X*R*** and a **Y*r*** chromosome failed to be disjoined in the reduction division, and **X*R*Y*r*** sperms were produced. The **X*R*Y*r*** sperms fertilizing **X*r*** ova produced **X*R*X*r*Y*r*** zygotes which developed into non-disjunctional males.

Considering those exceptional red males as the primary non-disjunctional individuals, we are now in a position to calculate their frequency. As the exceptional white females were not tested, as already mentioned, the red individuals only are to be taken into account. The total of the red females is 2563 and to them the non-disjunctional red males are as 1:360. In *Drosophila melanogaster* the total of the results of the experiments on primary non-disjunction obtained by BRIDGES, SAFIR, and MAVOR gives the ratio 1:2000 (MORGAN 1925), and the exceptional highest ratio among the results of SAFIR's experiments (SAFIR 1920) on five different stocks is 1:550. The ratio in our fish is about six times higher than the normal ratio in Drosophila and approaches near to the exceptional high ratio of SAFIR. Though, inasmuch as in Drosophila the the non-disjunction takes place in females, while in our fish it occurs in males, it will not be strictly rational to compare the two ratios, it is, how-

ever, apparent that in Aplocheilus the primary non-disjunction takes place much more frequently than in Drosophila.

SECONDARY NON-DISJUNCTIONAL MALES

The seven primary non-disjunctional red males mated to white females produced 1949 female offspring (some red, some white) and 19 male offspring (red and white, table 3). Of the 19 male offspring 3 were lost so that only the remaining 16 were genetically tested. They were all, except one, the red N, secondary non-disjunctional, and when mated to females of any kind—sisters, the wild form, or other color varieties—produced, like their male parents, mainly female offspring, but usually with a few male offspring. The latter were, with rare exceptions, also non-disjunctional. The results of the breeding tests of these males, generation after generation, are gathered in table 4.

The offspring of 16 sons (K, L, M, N, and $P_1 \cdots P_{12}$) of the primary non-disjunctional males are listed in the first part of the table. Among them the single one N produced, with wild brown females, 133 female and 156 male offspring (14B. 1926). This sex ratio is very close to the normal ratio 1:1 and the result of the breeding of offspring *inter se* in the next year showed also a normal sex ratio, so that N may safely be considered a normal male of the sexual constitution XY. This appearance of a normal male offspring is a convincing proof that the Y chromosome was present in their progenitor, the H male, and that the latter possessed Y in addition to two X chromosomes, as already mentioned.

The remaining 15 males were all secondary non-disjunctional of the first generation and produced in total 5535 offspring, of which 25 were male, the ratio being 200:1. The ratio in each culture varies between 0 and 10:1.

One male ($P_{3,2}$) of the 25 male offspring was an abnormal form unable to mate and yielded no offspring. The other 24 were all non-disjunctional and produced in total 4431 females and 81 males in the ratio of 55:1 (table 4, part 2). The production of males was about 3.5 times larger than in the first generation. The range of variation of the ratio in different cultures was naturally wider, extending from 0 to 7:1.

In 1927 I was able to rear offspring of the third generation of K-lineage (table 4, part III). Among 23 male offspring ($K_{1,1}$,$K_{2,1} \cdots K_{2,4}$,$K_{3,1} \cdots K_{3,4}$,$K_{6,1} \cdots K_{6,4}$, $K_{7,1}$, $K_{9,1}$, $K_{10,1} \cdots K_{10,8}$) of the secondary nondisjunctional males of the second generation, one male ($K_{2,1}$) was normal and yielded 106 female and 111 male offspring (2B. 1927). Another male ($K_{2,2}$) produced 57 female and 27 male offspring in the ratio 2:1

(3B. 1927). The number of the male offspring is here too great and the ratio approaches near to the most deviating sex ratio (3:2) ever found by me among my many years' breeding experiments on the normal individuals. So it will not be safe to consider $K_{2,2}$ as non-disjunctional, but the positive determination by its male offspring must be awaited until the next summer. At present, it is neglected in counting. Leaving aside these two individuals, the 21 other males produced a total of 3382 females and 185 males, the ratio being 20:1. The male ratio is here about thrice as large as that in the second generation and tenfold that in the first generation. The ratio in the different cultures varies between 0 and 4:1.

From all above stated, we see that the production of males in the offspring of non-disjunctional males increases exceedingly from generation to generation. What caused this increase — whether the presence of some gene or some local external condition—is not known at present. I hope that further investigation, now undertaken, will in the future throw some light upon this point.

The offspring of all non-disjunctional males, primary and secondary, consists, in total, of 15,272 females and 310 males. Among the males 63 were genetically tested, and only two were found to be normal, and the remaining 61 were all non-disjunctional. From these facts we may conclude that the non-disjunctional males of *Aplocheilus latipes*, either primary or secondary, produce chiefly female offspring and few non-disjunctional males, so that the lineage of non-disjunction may be perpetuated simply by breeding the male offspring with any normal female from generation to generation. The production of normal males was very rare; 2 out of 65 males were normal.

Since hitherto I have been unable to engage in cytological investigation, no evidence has yet been obtained about the behavior of the non-disjunctional chromosomes. It is however highly probable that in the reduction division of the non-disjunctional male the homosynapsis of two X chromosomes normally takes place among the three chromosomes X, X, and Y, and that at the stage of the separation of chromosomes to two poles the superfluous solitary Y chromosome lagging in the middle of the spindle might be excluded from the formation of the gametic nuclei. Owing to such a process, all gametes of the non-disjunctional male parent might have one X chromosome and consequently only females be produced. In some cases, the Y chromosome may enter with the X chromosome into one of the nuclei and form a gamete possessing X and Y chromosomes, which will produce secondary non-disjunctional males. The heterosynapsis XY may also occur but very rarely. In this case, one X is the lagging

chromosome and is generally eliminated, and consequently two gametes are produced, one having the X and the other the Y chromosome. The gamete possessing Y will produce the normal male offspring. The total number of the offspring of the non-disjunctional males whose male offspring were genetically tested was 10,274 (the sum of 7 cultures of primary non-disjunctional males and the cultures of secondary non-disjunctional males in 1925 and 1926), out of which only two were normal males. But the heterosynapsis will not be the sole way of the production of normal male. The elimination of X in the course of the development of XXY ovum will also produce a normal male. The red N male is the offspring of the red non-disjunctional male $XRXrYr$ mated to white female $XrXr$. Heterosynapsis between XR, Xr and Yr chromosomes is unable to produce R carrying sperm which will produce, with an X ovum, a normal male unless R crosses over from X to Y chromosome. It is more reasonable to conjecture the formation of an N individual by elimination of one Xr in $XRXrYr$ ovum than to suppose a concurrent occurrence of crossing over and rare heterosynapsis. If the synapses in an XXY male takes place at random, the number of XY synapses should be twice as great as tha' of XX synapses and one third of the whole number of gametes would receive the disjoined Y chromosome and produce a normal male, except in a few cases where it is accompanied by the lagging chromosome. The scarcity of normal males shows that there must be a strong preference for homosynapsis. It is exceptionally strong in our fish and much stronger than in *Drosophila melanogaster*, where BRIDGES (previously cited) has observed homosynapsis 83.5: heterosynapsis 16.5. In Aplocheilus, indeed, the frequency of the occurrence of heterosynapsis in non-disjunctional males may be well conceived roughly to be zero.

This strong perference for homosynapsis with the preponderance of one-sided crossing over, as already mentioned, seems to indicate some morphological or physiological difference between the X and Y chromosomes in our fish, and does not support the supposition of WINGE that X and Y chromosomes in Lebistes are homologous, differing only in the sex-determining genes contained.

NEW MUTANTS, "FUSED" AND "WAVY"

Some aberrant types of the vertebræ appeared from time to time in our stocks. Sometimes the vertebral bodies anchylose together, and when the anchylosis takes place in many vertebræ, the vertebral column is much shortened, and the fish shows a dwarf form. In other cases, the vertebral column curves wavily, dorso-ventrally, and shows in side view

a high or low undulation. It also curves in some fishes wavily laterally, or sometimes bends the caudal portion simply to the left or right side. Occasionally, only the terminal vertebra bends laterally and makes a small prominence at the base of the caudal fin. Breeding tests show that the two last mentioned aberrations are not inherited and may be looked upon as developmental abnormalities. The former two, "fused" and "wavy," are heritable mutants, and some description of them will here be given.

"Fused." This is a character recessive to the normal and independent of all known color genes and of sex. It varies in its intensity somewhat widely, and, in an extreme case, nearly all the vertebræ fuse into a few pieces, so that the fish shows a strong dwarfishness. Such a form may easily be distinguished externally from the normal even in an early stage of its growth. When only a few vertebræ fuse, the character is impossible to detect externally, and can be recognized only after examination by dissection.

This mutant is much less viable than the normal. Especially when it is cultured together with the normal, its death rate is greatly increased. Hence, in counting it in a culture, I first selected the individuals which were conspicuously dwarf and in which the fusion takes place a few weeks after hatching. These were included in a preliminary count. The remaining fishes were cultured till they grew nearly to their full size, and their vertebrae were examined after dissection. The number of the mutants then obtained was added to the first count, and the final results were thus obtained. As the death of some individuals is inevitable in a culture which is continued about two months in order to let the fish reach their full size, naturally the number of the mutants obtained is much less than the expectation.

The F_1 offspring of fused mated to the normal were normal, and of the F_2, 3212 were normal and 988 fused. In a back cross with fused, the heterozygous F_1 produced 837 normal and 750 fused.

"Wavy." The vertebral column in this mutant curves dorso-ventrally and forms generally two wavy crests. The external body form shows a more or less wavy outline dorsally as well as ventrally, according to the undulation of the vertebral column. This character is also recessive to the normal and independent of all other known genetic characters and of sex. It was found more than once in our stocks, especially in the heterozygous fused stock, but the breeding tests showed no linkage between it and "fused."

The F_1 offspring of "wavy" mated to normal were all normal and F_2

was composed of 1528 normals and 469 wavies. In back cross with "wavy," the F_1 yielded 643 normals and 503 wavies. These results do not fit strictly the expected proportions of 3:1 and 1:1, but as they were cultured in mass, the weak "wavy" together with the strong normal, differential viability might be expected, and so, a deficiency of "wavy" individuals.

In pure matings of these two mutants, a few individuals have reverted to the normal form. One out of 70 fused, and one out of 80 wavies were in form normal, and both were heterozygous.

In other species of fish, I have observed rarely abnormal forms similar to the above mentioned. The fused type was found in *Pagrus tumifrons* T. and S., *Cyprinus carpio* L., and *Carassius auratus* (L.); and the wavy type in *Cyprinus carpio* L. and *Plecoglossus altivelis* T. and S. These abnormalities seem to be widely spread among fishes.

Summary

The inequality of the frequencies of transfer of a dominant gene from the Y to the X chromosome and from the X to the Y chromosome is described. The *R* gene, which is responsible for the formation of yellow pigment, crosses over from Y to X with a frequency of 1:300, while it returns from X to Y with a frequency of 1:1200. There is a large preponderance of the crossing over from Y to X rather than from X to Y.

The non-disjunctional form XXY in *Aplocheilus latipes* is male. It produces with any normal female mainly female offspring, with a few non-disjunctional males, or none.

The frequency of the occurrence of primary non-disjunction is 1:360. The frequency of secondary non-disjunction increases with each successive generation; at the third generation it was tenfold that of the first generation.

Two recessive mutants, "fused" and "wavy," are described.

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AN EXPERIMENTAL STUDY OF HYBRID VIGOR OR HETEROSIS IN RATS[1]

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INTRODUCTION

A review of the literature of both plant and animal hybridization shows that increased vigor of growth and reproduction usually accompanies hybridization although it is by no means a universal phenomenon. The term hybridization is here used as it is commonly employed by students of genetics rather than in the restricted sense sometimes ascribed to it in systematics. It applies to any cross between individuals of different genetic constitution, whether or not they belong to different Linnean species, or merely to different varieties, breeds or families of the same species.

HYPOTHESES ADVANCED AS AN EXPLANATION OF HYBRID VIGOR

The fact that the early plant and animal hybridizers did not know of any mechanism of heredity accounts for their failure to attempt to give any explanation as to the cause of hybrid vigor. Animal breeders, however,

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did make use of both inbreeding and crossbreeding in the early development of many breeds of farm animals. They realized that while close-breeding tended to standardize type, such a system of breeding often concentrated undesirable characters, such as loss of vigor and size, low fertility and susceptibility to disease. Their antidote for these bad conditions was the use of an unrelated sire, and as a rule more vigorous and productive offspring were the result of such an outcross.

DARWIN (1877) could not see why the bad qualities of inbred plants and animals could be caused by a concentration of such qualities, because he knew of many cases of inbred plants and animals of different strains or coming from different herds but possessing the same or similar undesirable qualities, which when crossed, gave vigorous progeny. Cases of this kind proved to him the great advantage of crossing even though the individuals crossed were weak or carried undesirable qualities. He attributed the hybrid vigor to the bringing together of diverse sexual elements from compatible but somewhat unrelated types, and not to the mere act of crossing. His closing arguments on the value of cross-fertilization are based on the fact that there must be a difference in the sexual elements before hybrid vigor is possible. DARWIN believed that the environment of individual plants or animals was directly associated with the change in, or the differentiation of the sexual elements. He seems to have been the first to offer an explanation of hybrid vigor.

DAVENPORT (1908) spoke of the influence of dominant and recessive factors in a pair of Mendelian allelomorphs. He believed the dominant factor to be the progressive one while the recessive was retrogressive and often lacked in vigor. From this point on, two general hypotheses of hybrid vigor have been advanced.

In brief, one of these hypotheses is based upon the degree of heterozygosity brought about by crossbreeding, that is, the more nearly crossbreeding brings about a 100 percent heterozygous condition of all allelomorphic pairs of genes the more vigorous the hybrid will be. This conception of hybrid vigor led to the acceptance of "heterosis" (G. H. SHULL) as a logical word to express the increased growth or vigor possessed by many hybrid animals and plants. The second of these hypotheses is based upon the number of dominant genes which are brought together in hybrids and the greater the number of dominants, it is supposed, the more clearly will hybrid vigor be manifested. A modification of the second hypothesis regards certain dominant genes as contributing more largely than others to the vigor of the offspring, or even as having a predominant influence on vigor so that they can be regarded as specific "growth genes."

Three investigators seem to have been interested in the dominant hypothesis as an explanation of hybrid vigor at about the same time. BRUCE (1910) points out that the total number of dominant factors is greater in a hybrid population than in either parent population and that there is consequently a correlation between hybrid vigor and the number of dominant factors. He did not state why a greater number of dominant factors produced an increase in vigor. KEEBLE and PELLEW (1910) believed that the dominance of characters contributed by both parents might explain the increased vigor of hybrids. They crossed two varieties of peas (*Pisum sativum*) which differed in height and the F_1 hybrids were taller than either parent. The dominance of characters in the hybrids was offered as a possible explanation. I am not able to state whether the first suggestion of the dominant hypothesis should be credited to BRUCE or KEEBLE and PELLEW, but G. H. SHULL (1911) gives the credit to BRUCE.

In discussing "The Genotypes of Maize" SHULL mentions two possible explanations of hybrid or crossbred vigor. The first, and the one which SHULL favored, gave heterozygosis credit for the increased vigor and size of the F_1 generation. In many corn crosses which gave greater vigor to the F_1 hybrids he believed that a correlation existed between the degree of vigor and the number of heterozygous characters. To quote, "I do not believe that this correlation is perfect, of course, but approximate, as it is readily conceivable that even though the general principle should be correct, heterozygosis in some elements may be without effect upon vigor, or even depressing." The second hypothesis mentioned by SHULL, for which he gives A. B. BRUCE credit, is based on the assumption that the degree of vigor depends upon the number of dominant elements present rather than the number of heterozygous elements. The following quotation explains SHULL's reaction to the two hypotheses: "Mr. A. B. BRUCE proposes a slightly different hypothesis in which the degree of vigor is assumed to depend upon the number of dominant elements present, rather than the number of heterozygous elements. While all of my data thus far are in perfect accord with my own hypothesis, and I know of no instance in which self-fertilization of a corn plant of maximum vigor has not resulted in a less vigorous progeny, it is quite possible that I have still insufficient data from which to distinguish the results expected under these two hypotheses. However, for the purpose of the present discussion, it is not necessary to decide which of these two hypotheses (if either) is correct. Both of them are based upon the view that the germ-cells produced by any plant whose vigor has been increased by crossing are not uniform, some possessing positive elements or genes not possessed by others."

EAST and HAYES (1912) give support to the heterozygosis hypothesis in reporting their results secured in tobacco crosses. These investigators make a clear distinction between dominance and the effects of heterozygosis. The following quotation is of value in order to understand fully their support of the hypothesis of heterozygosis: "The term vigor has hitherto been used with the general meaning which the biologist readily understands. We will now endeavor to show in what plant characters this vigor finds expression. It is not an easy task because of the possibility of confusing the phenomena of Mendelian dominance with the physiological effects due to heterozygosis. The confusion is due to superficial resemblance only. Dominance is the expressed potency of a character in a cross and affects the character as a whole. A morphological character, like the pods of individual maize seeds, or the products of some physiological reaction like the red color of the seed pericarp in maize, may be perfect dominance, that is, it may be developed completely when obtained from only one parent. Size characters, on the other hand, usually lack dominance or at least show incomplete dominance. The vigor of the first hybrid generation theoretically has nothing to do with these facts. This is easily demonstrated if one remembers that the increased vigor manifested as height in the F_1 generation can not be obtained as a pure homozygous segregate, which would be possible if due to dominance. Furthermore, the universality with which vigor of heterozygosis is expressed as height shows the distinction between the two phenomena. If the greater height were the expression of the meeting of two factors ($T_1t_2 \times t_1T_2$) both of which were necessary to produce the character, one could not account for the frequency of the occurrence. Nevertheless, in practice the confusion exists, and while we have considerable confidence in the conclusions drawn from our experiments we have no intention of expressing them dogmatically."

A. F. SHULL (1912) and CASTLE (1916) agree that heterosis cannot be explained satisfactorily on the basis of dominant factors. SHULL, although not taking a definite stand for either hypothesis, does state that heterozygosis is more likely to account for hybrid vigor than the presence of certain dominant genes. To quote SHULL: "The former view (heterozygosis) admits of a plausible foundation in cell physiology, and the essence of it may be extended to cases of decrease of vigor in which there is no change in genotypic constitution and which are, therefore, without the pale of either theory." CASTLE takes somewhat the same view as SHULL as will be noted in the following quotation: "Crossbreeding has, then, the same advantages over close-breeding that fertilization has over

parthenogenesis. It brings together differentiated gametes, which, reacting on each other, produce greater metabolic activity. Whether or not the uniting gametes differ by Mendelian unit-characters is probably of no consequence. That they differ chemically is doubtless the essential thing in producing hybrid vigor. Heterozygosis is mentioned merely as an evidence of such chemical difference."

EMERSON and EAST (1913) offer an objection to the hypothesis of dominance because F_2 generations do not show an unsymmetrical distribution in respect to characters in which heterosis was shown in the F_1. This objection was based upon their study of the inheritance of quantitative characters in maize.

JONES (1917) revived the dominance hypothesis by introducing linked factors as an explanation as to why it was very difficult to get homozygous segregates possessing the vigor so often manifest in F_1 hybrids. The knowledge that genetic linkage often occurs does in a measure answer one of the chief objections to the dominance hypothesis. This principle of linkage also answers the objection raised by EMERSON and EAST as to why F_2 generations do not show an unsymmetrical distribution in respect to many known characters. A chief difficulty of the hypothesis is the necessary supposition that genes favoring growth are so specific in nature and so few in number that their being borne on homologous chromosomes and at adjacent loci in different races of the same species could regularly result in repulsion in gametogenesis in F_1 individuals, so that hybrid vigor regularly disappears in F_2. JONES (1918) discussing "a Mendelian interpretation of heterosis" makes the following statements:

"Whether or not dominance of factors is wholly adequate to account for all of the immediate effects of exogamy remains to be seen. The former view that dominance was not concerned at all has been maintained so insistently that I have taken the extremely opposite view in order to show that dominance at least can be held responsible for a large part of the increased development shown by hybrids. The treatment of the subject in this light has been dogmatic. That cross-fertilization may produce some effect which can never be attained in self-fertilization or asexual reproduction is still possible.

"The difference between the two hypotheses is not as great as might seem at first sight. The older hypothesis is general in its application and does not commit itself to the interpretation of specific effects. The view presented here is specific in its application and may be shown to be inadequate for the interpretation of all phases of the problem of increased development following cross-fertilization.

"The greatest progress in our knowledge of inbreeding and crossbreeding was made when their effects were linked with Mendelian phenomena. This was the big step forward. The two ways of interpreting these results here differ only in minor features and it is not putting the matter fairly to hold them up as rival hypotheses, one to be chosen from the other. Placing the effects of inbreeding and crossbreeding entirely on a Mendelian basis is merely an outgrowth of the older view as knowledge of the methods of inheritance increased."

EAST and JONES (1919) agree that the dominance hypothesis explains heterosis, and state that the recent developments in our knowledge of hereditary factors, especially linkage conditions, remove the major objections to such a hypothesis. In other words, EAST has abandoned the heterozygosis hypothesis in favor of the dominance hypothesis. Hayes has also been converted to the latter hypothesis as indicated in the text on "Breeding Crop Plants" by HAYES and GARBER (1927).

WRIGHT (1922) makes the following statements in connection with results of inbreeding and crossbreeding of guinea pigs:

"Analysis of the various crosses indicates that the results are all the direct or indirect consequence of the Mendelian mechanism of heredity. The fundamental effect of inbreeding is the automatic increase in homozygosis in all respects. An average decline in vigor is the consequence of the observed fact that recessive factors, more extensively brought into expression by an increase in homozygosis, are more likely to be deleterious than are their dominant allelomorphs. The differentiation among the families is due to the chance fixation of different combinations of the factors present in the original heterozygous stock. Crossing results in improvement because each family in general supplies some dominant factors lacking in the others. Dominance or even imperfect dominance in each unit character is built up into a pronounced improvement over each parent stock in the complex characters actually observed.

"A certain portion of the increase in vigor of the first cross between inbred families is maintained on resuming random-mating. One half of this increase is maintained in stocks founded on two inbred lines, two-thirds in the case of three lines, three-fourths in the case of four lines, four-fifths in the case of five lines, and so on." The above quotations leave no doubt where WRIGHT stands on the two hypotheses.

DISCUSSION OF THE HYPOTHESES OF HYBRID VIGOR

The essential difference between the explanations which have been offered for hybrid vigor seems to be this: On the heterosis interpretation

hybrid vigor does not result either from dominant or from recessive genes as such, but from a union of unlike elements dominant with recessive, *dissimilarity* in content of genes between the uniting gametes producing, it is supposed, a more vigorously developing zygote.

The original suggestion that a greater content of *dominant* genes made for greater vigor seems to have been a logical outgrowth of the presence-absence concept, in which recessives were conceived as simple absence of the dominants. Naturally if a dominant contributed anything to the total vigor of the organism, the recessive would contribute less, so that, other things being equal, the most vigorous zygote would be the one containing the most dominants.

In the revival of the dominant theory by JONES, the presence-absence idea is dropped, and the conception brought forward of special (dominant) genes making for vigor in the organism. If a combination of all such genes could be secured in a homozygous state the maximum of vigor would be realized, according to JONES, and a heterozygous state of one or more such genes would add nothing to the vigor of the organism. In a recent paper (1926) he cites the KING inbred rats as an example of a presumably, completely homozygous organism possessing a maximum of vigor. Experimental observations to be presently discussed show, however, that still further vigor of development can be imparted to the KING inbred race by an outcross with a race no larger than itself.

In every such case JONES is forced to assume that the race with which a cross was made did after all contain one or more genes making for vigor which were not present in the inbred race. He might then simply say that he was mistaken in his previous estimate of the KING race. It left something yet to be desired in the way of vigor. The question is whether there has ever existed, or can be produced, an organism which would not derive additional vigor from an outcross. This is the gist of the heterosis interpretation, for or against which experimental evidence is desired.

EXPERIMENTAL STUDY OF HYBRID VIGOR

Location of Work and Animals Used

Experimental work was started at the Bussey Institution, HARVARD UNIVERSITY, Forest Hills, Boston Massachusetts in the fall of 1924. After one year's work at the Bussey Institution all necessary experimental animals were moved to the WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION, Morgantown, West Virginia, where active breeding was continued until the late summer of 1927.

Three strains of rats (*Rattus Norvegicus*) were used and for convenience in keeping records they were designated as strains S_1, S_2, S_3. Such symbols are used exclusively in charts, tables, and in all discussions referring to the different strains. These strains were stock rats maintained at the Bussey Institution laboratory previous to their use in my work.

The S_1 strain may be described as pink-eyed yellow rats of the known genetic composition *CCppRRHHAA*. This stock had been rather closely bred for several generations although sib (brother and sister) matings had not been regularly followed. Ten rats of this strain, 8 females and 2 males, were selected from two litters for my work. These two litters were from sib matings, having the same sire and their dams being litter mates to each other and to the male. This strain proved to be vigorous and prolific throughout the experiment.

The rats of the S_2 strain are red-eyed hooded cream with the known genetic composition *CCPPrrhhaa*. This strain had been more closely inbred than the S_1 strain, having been closely inbred for several generations. The matings were known to have been sib matings for three generations. This strain was known not to be very prolific and it was necessary to select my initial animals, nine in number, from three litters. Two females and one male were selected from each litter. This strain was a handicap to the progress of the work due to small litters and also due to the failure of a number of females to produce more than one litter. I am unable to explain the fact that many females of this strain produce one litter and fail to conceive thereafter.

The rats of the S_3 strain are known as KING inbred albinos. This strain was secured from the WISTAR INSTITUTE of Philadelphia through the kindness of Doctor HELEN DEAN KING, a short time before this experiment was started. The known genetic composition of this strain is *ccPPRRhhaa*. The foundation rats of this strain consisted of six females and one male from a litter of twelve. This litter represented the fifty-first generation of brother by sister matings according to the records of Doctor KING and the Bussey Institution. This strain proved prolific and vigorous throughout the experiment.

Basis of Study

This study of hybrid vigor is based solely upon growth as measured by weight. A careful growth record was made of all stock strains (S_1, S_2, and S_3) and of the F_1 and F_2 progeny of crosses, $S_1 \times S_2$ and $S_1 \times S_3$. The F_2 progeny of the $S_1 \times S_3$ cross were all tested for their genetic consti-

tution by back-crossing to the stock strains, thus giving a known genetic basis for studying animals of the F_2 generation.

Methods of Handling Experimental Animals

1. Rats were kept in the standard $14'' \times 17''$ wire cages which were used for all rat work at the Bussey Institution.
2. All rats were fed and cared for in as like a manner as possible.
3. With few exceptions, five rats were retained in a cage from the time they were weaned at 30 days of age until they were 90 days of age.
4. Cages and other equipment (water bottles and feed dishes) were thoroughly cleaned each week to guard against disease and digestive disturbances.
5. All rats were ear marked and careful growth weights to the nearest 5 grams were recorded for 30, 50, 70, 90 and 150 days of age.
6. A Hanson Brothers' spring scale, graduated to one gram and weighing a maximum of 500 grams, was used for weighing the rats.
7. All stock rats (S_1, S_2, and S_3) were produced by brother-sister matings during the experiment.
8. In all cases total litters were recorded and the only rats born in litters contained in this work, not accounted for, died before their growth records were complete, or failed to breed. Apparent sterility in a few F_2 ($S_1 \times S_3$) rats eliminated them from studies involving their genetic constitution.
9. All statistical studies are based on the weights of rats at 90 days of age. Many females were mated following the 90 day weight period and the subsequent occurrence of gestation and lactation periods, change of cages, and the varying number of animals kept in one cage make it undesirable to use the weights of the 150 day period.

DATA AND STATISTICAL STUDIES

Average Weights

Males and females of each parent strain and of their F_1 and F_2 progenies were treated separately to get the average weight of each sex at 30, 50, 70, 90, and 150 days of age. Table 1 shows these average weights and the number of rats each group contains. This table does not contain all rats used in this study, as many stock rats were used for breeding purposes in connection with tests to determine the genetic constitution of F_2 ($S_1 \times S_3$) rats. For such test animals growth weights were not recorded. The S_2 strain of rats gave so much trouble, due to small litters and their failure

to breed, that the progeny of the cross between the S_1 and S_2 strains were not studied extensively. However, the data derived from this cross are of interest in connection with the data of the other cross ($S_1 \times S_3$). The data contained in table I are given graphically in figures 1–4.

TABLE 1

Average weight in grams of rats of various stocks and of their F_1 and F_2 progeny at ages 30–150 days.

STOCK	SEX	NO	30 DAYS	50 DAYS	70 DAYS	90 DAYS	150 DAYS
S_1	Males	100	43	86	132	168	234
S_1	Females	98	44	81	111	134	175
S_2	Males	52	30	58	100	130	189
S_2	Females	59	28	54	79	100	137
S_3	Males	62	46	99	158	199	268
S_3	Females	80	45	87	125	153	199
$F_1(S_1 \times S_2)$	Males	48	47	101	159	206	272
$F_1(S_1 \times S_2)$	Females	37	44	92	129	156	191
$F_2(S_1 \times S_2)$	Males	51	46	97	150	183	247
$F_2(S_1 \times S_2)$	Females	64	42	83	116	141	179
$F_1(S_1 \times S_3)$	Males	53	44	117	179	213	278
$F_1(S_1 \times S_3)$	Females	69	43	97	141	161	205
$F_2(S_1 \times S_3)$	Males	137	42	87	133	177	244
$F_2(S_1 \times S_3)$	Females	132	41	81	114	142	179

Growth Curves

By a glance at figures 1 and 3, one is convinced that heterosis is exhibited in F_1 animals of each cross made. Figures 2 and 4 show that this F_1 vigor is materially reduced in the F_2 generation of each cross. In the case of the S_1 by S_2 cross (figure 1) there is a large difference in size between the parent strains. The average weight of S_1 males is 234 grams and the average weight of S_2 males is 189 grams at 150 days of age, yet the F_1 males have an average weight greater than that of males of the heavier parent race (S_1), namely 272 grams at 150 days of age. The same relation exists in the case of the F_1 females compared with the parent stocks. Figure 2 shows that both the F_2 males and the F_2 females fail to develop as rapidly and are smaller at 150 days of age than the same sex of the F_1 generation. Even though a noticeable decrease in size is shown in the F_2 generation, both males and females are still heavier at 150 days of age than the males and females of the larger or S_1 parent strain. In

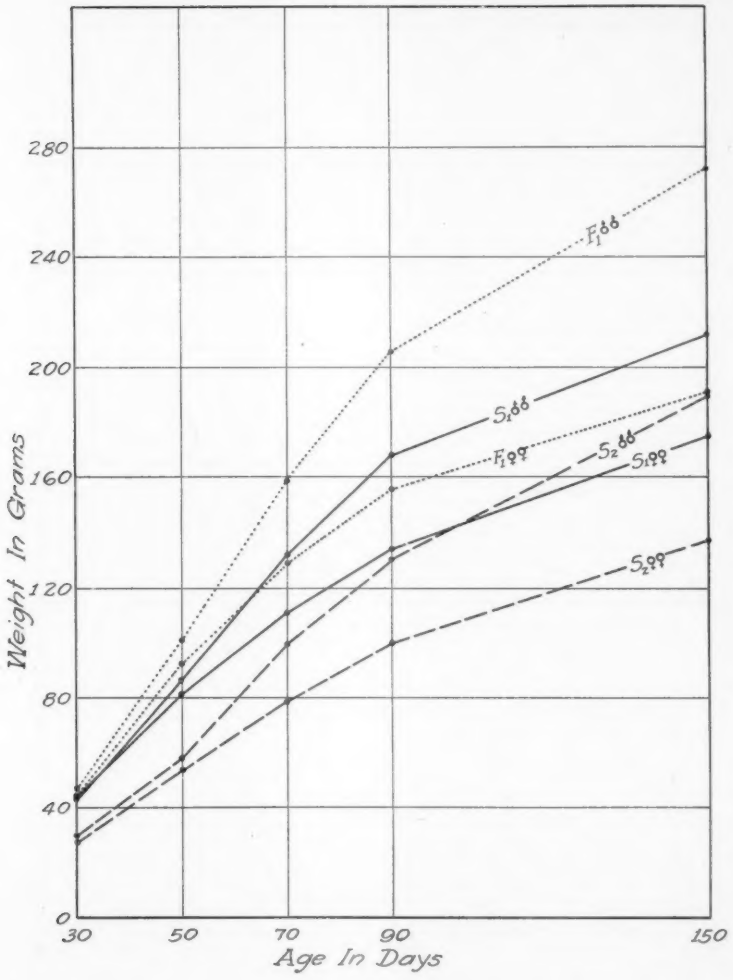

FIGURE 1.—Growth curves for S_1 and S_2 stock and their F_1 progeny.

this cross S_2 males were, in all cases, mated to S_1 females due to the low productiveness of the S_2 females.

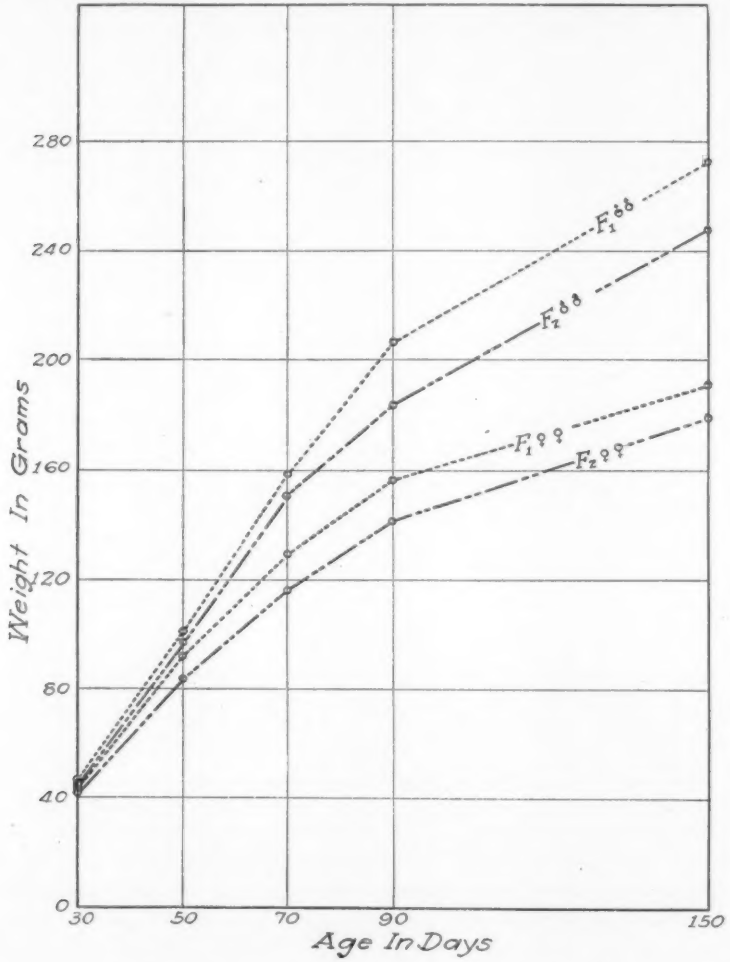

FIGURE 2.—Growth curves for F_1 and F_2 progeny of $S_1 \times S_2$ stocks.

The stock strains of the S_1 by S_3 cross, as shown in figure 3, are nearer the same size at 150 days of age than were the stock strains (S_1 and S_2)

of the previous cross. The results of the S_1 by S_3 cross are comparable to the results of the S_1 by S_2 cross, with one exception; the F_2 generation

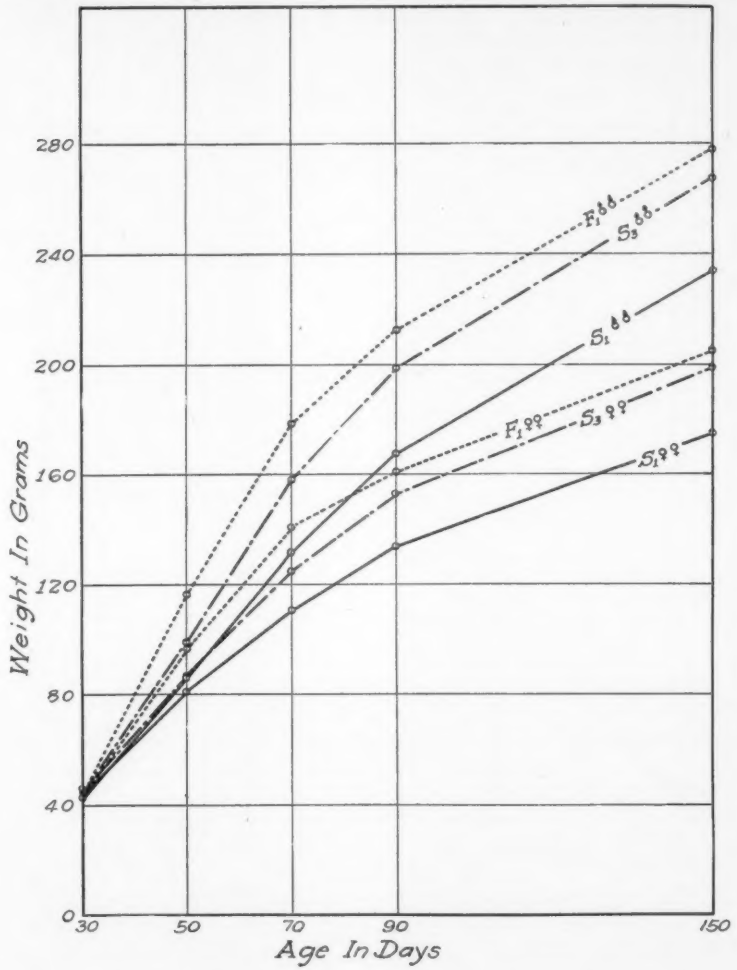

FIGURE 3.—Growth curves for S_1 and S_3 stock and their F_1 progeny.

(figure 4) drops in average weight to near the average weight of the smaller or S_1 parent strain at the same age. In this case the F_1 vigor apparently

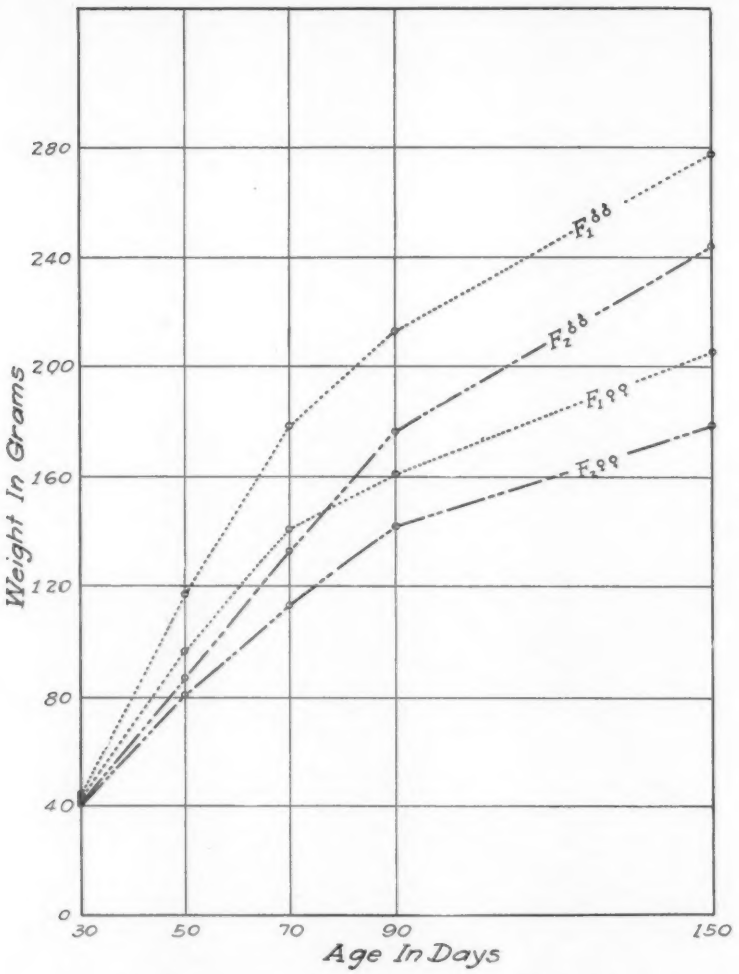

FIGURE 4.—Growth curves for F_1 and F_2 progeny of $S_1 \times S_3$ stock.

was being lost at a more rapid rate than in the former cross. The average loss in weight between the F_1 and F_2 generations, at 150 days of age, in the two crosses is as follows:

	$S_1 \times S_2$	$S_1 \times S_3$
Loss in weight of males	25 grams	34 grams
Loss in weight of females	12 grams	26 grams

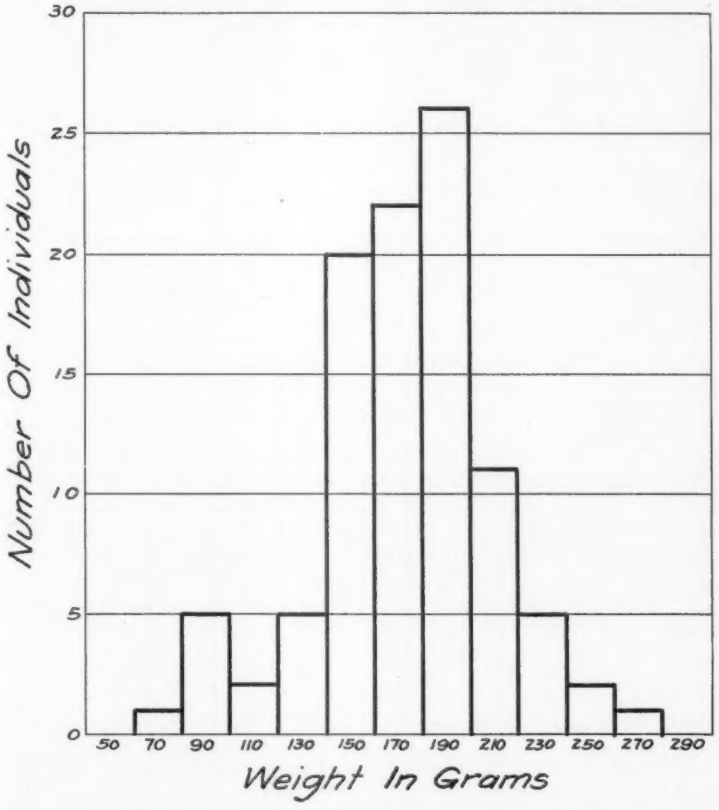

FIGURE 5.—Frequency polygon showing variation in weight of 100 S_1 males at 90 days of age.

Frequency Polygons

Frequency polygons (figures 5–12) show the variations in weight at ninety days of age of all male and all female rats in connection with the

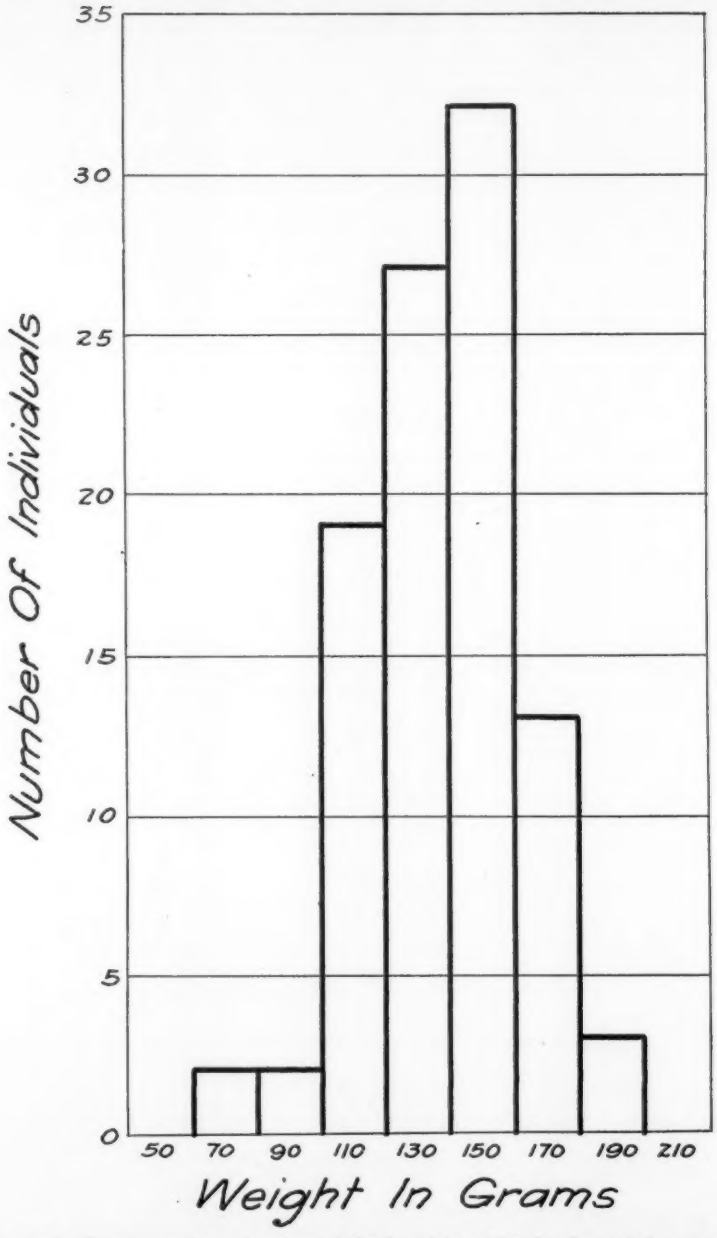

FIGURE 6.—Frequency polygon showing variation in weight of 86 S_1 females at 90 days of age.

$S_1 \times S_2$ cross. These graphs show that male rats are more variable than females in the S_1 and S_2 stock strains. The F_1 and the F_2 rats do not show any marked difference in the variablility of males and females. However, table 2 shows that the coefficient of variability for the F_1 females is slightly higher than for the F_1 males, and that the coefficients of variability of the F_2 males and females are almost identical. Figures 9 and 11 show slightly more variability among the F_2 males than among the F_1 males,

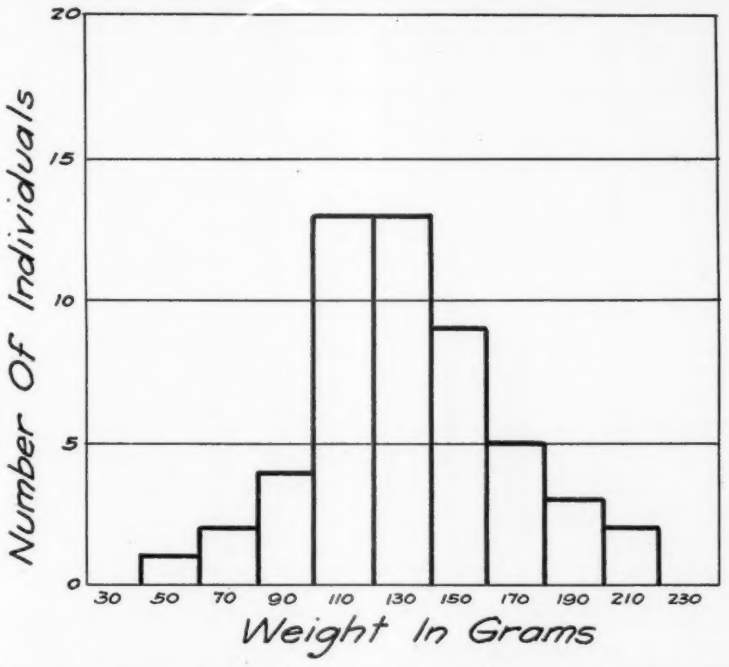

FIGURE 7.—Frequency polygon showing variation in weight of 52 S_2 males at 90 days of age.

but the F_2 males are less variable than the males of either parent strain (figures 5 and 7). Figures 10 and 12 do not indicate a greater variability among the F_2 females than among the F_1 females. In fact, these graphs indicate less variability among the F_2 females. Table 2 shows the coefficients of variability for the F_1 and F_2 females to be almost identical.

Frequency polygons (figures 5, 6, and 13–18) show the variations in weight of all male and all female rats in connection with the S_1 by S_2

cross, at 90 days of age. These graphs indicate that male rats are uniformly more variable than females of the same stock strain or the same progeny generation. This greater variability of the body weight of male rats was observed also by KING (1923). These graphs also indicate that

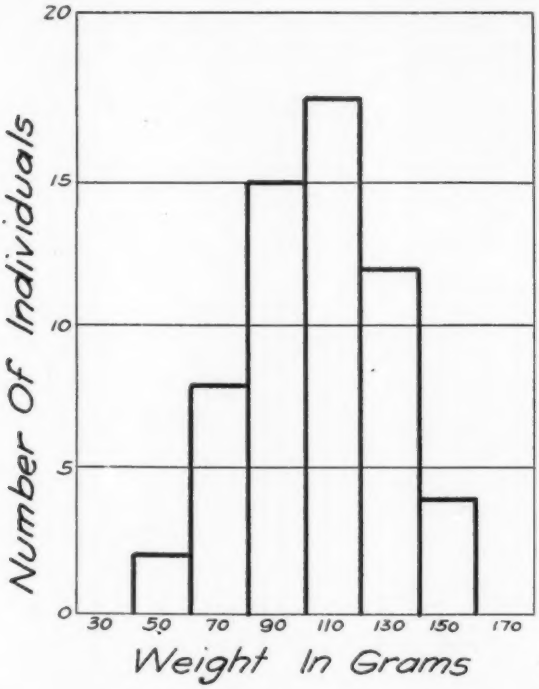

FIGURE 8.—Frequency polygon showing variation in weight of 59 S_2 females at 90 days of age.

both F_1 males and F_1 females are less variable than the corresponding sex of either parent strain. Figures 15 and 17 indicate a greater variability among F_2 males than among F_1 males, but this relation does not exist in the case of F_1 and F_2 females as shown in figures 16 and 18.

Statistical Study at Ninety Days of Age

Growth curves and frequency polygons are valuable graphical helps to visualize the comparative rate of growth and the comparative variations of the groups of rats studied, but they do not give a positive answer as to whether the difference in growth or the difference in variation is large

enough to be significant. Therefore a more reliable statistical study has been attempted as shown in tables 2, 3, and 4. The data contained in table 2 are based upon the weights of rats at ninety days of age. I have previously mentioned why I selected weights at this age for this study.

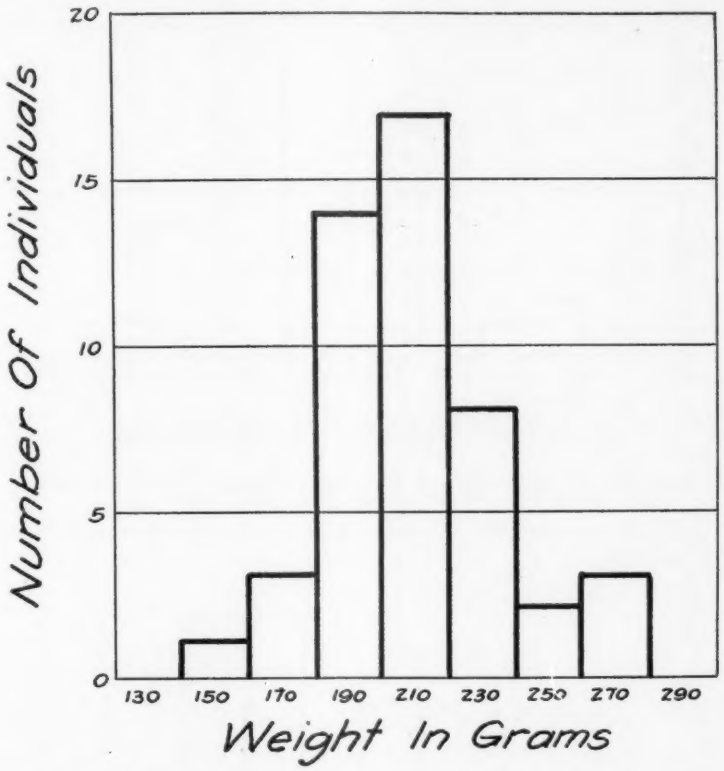

FIGURE 9.—Frequency polygon showing variation in weight of 48 F_1 ($S_1 \times S_2$) males at 90 days of age.

The data of table 2 were calculated on the same frequency as previously shown in frequency polygons.

Tables 3 and 4 are based on the data contained in table 2 and show in a comparative way whether the differences in mean weights and the differences in coefficients of variability are significant or not. In making these

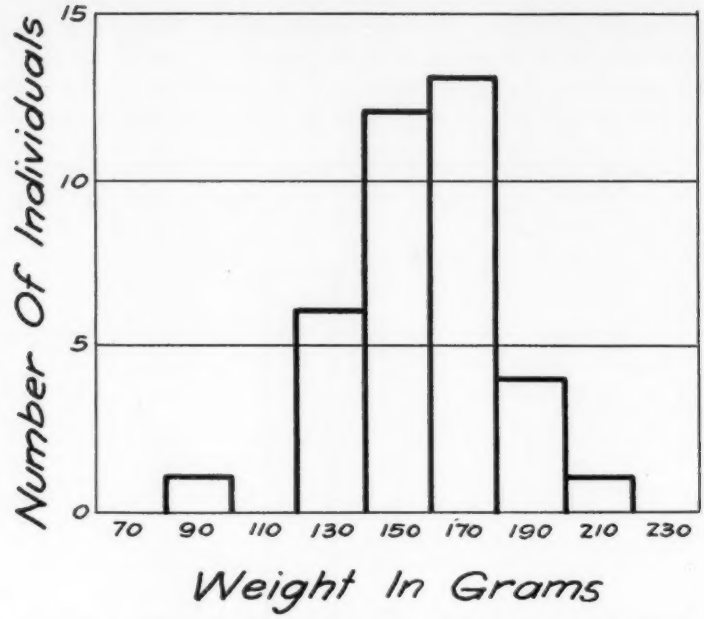

FIGURE 10.—Frequency polygon showing variation in weight of 37 F_1 ($S_1 \times S_2$) females at 90 days of age.

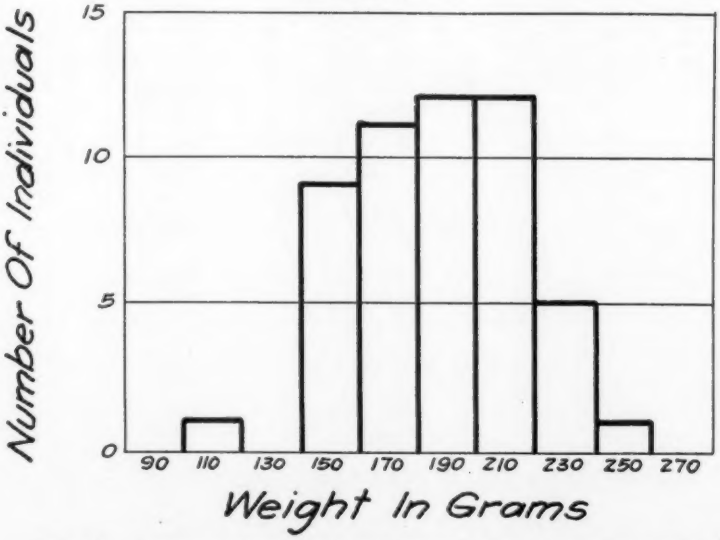

FIGURE 11.—Frequency polygon showing variation in weight of 51 F_2 ($S_1 \times S_2$) males at 90 days of age.

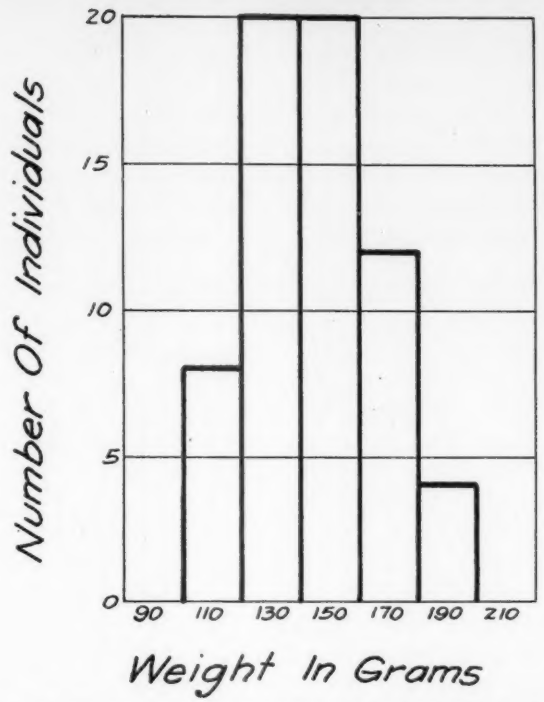

FIGURE 12.—Frequency polygon showing variation in weight of 64 F_2 ($S_1 \times S_2$) females at 90 days of age.

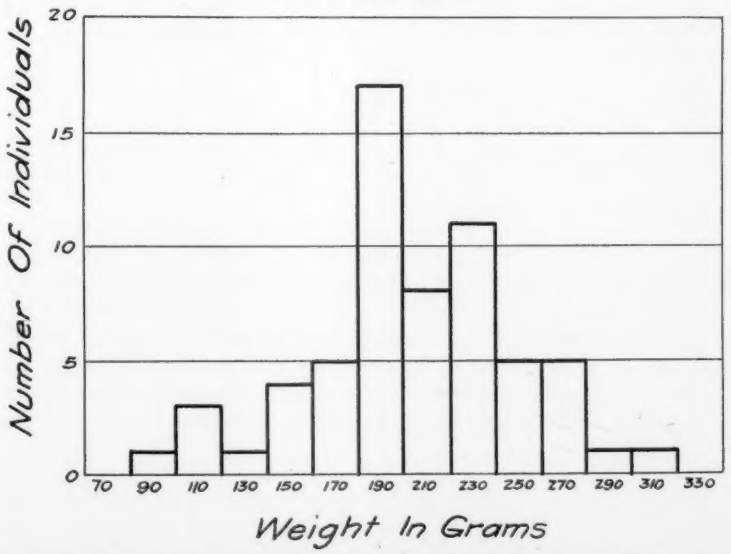

FIGURE 13.—Frequency polygon showing variation in Weight of 63 S_3 males at 90 days of age.

comparisons I used the following formula in calculating the probable error of the differences:

$$\text{Probable error of difference} = \sqrt{\Sigma(\text{p.e.})^2}$$

Table 3 contains the comparative data on the difference in mean weights and the difference in the coefficient of variability of groups of rats connected with the $S_1 \times S_2$ cross. These data show that both the S_1 males and the S_1 females are significantly larger than the same sex of the S_2 strain;

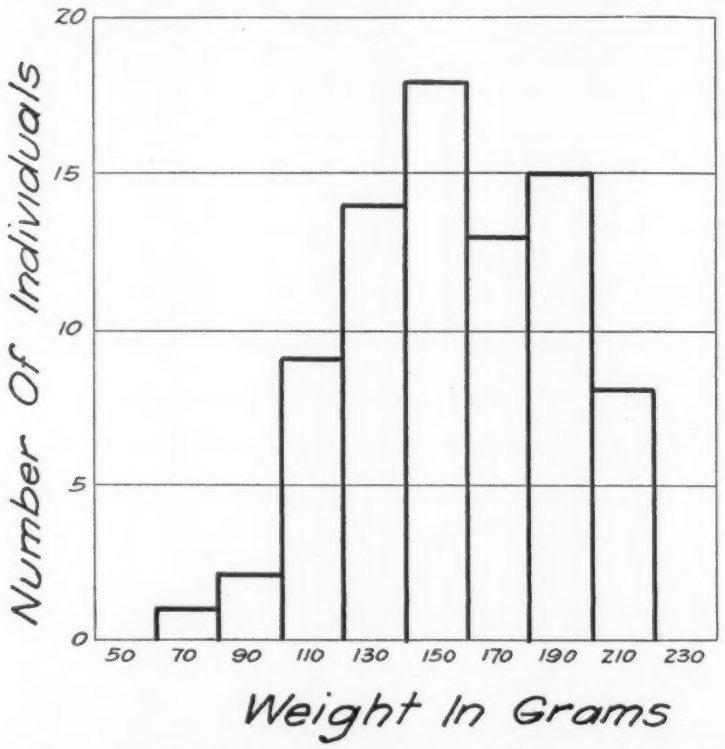

FIGURE 14.—Frequency polygon showing variation in weight of 80 S_3 females at 90 days of age.

that the S_2 females are significantly more variable than the S_1 females; and that while the S_1 males are more variable than the S_2 males, it is not a significant difference. In comparing the S_1 and S_2 strains with the F_1 generation we find both males and females of the F_1 generation signifi-

cantly larger and less variable than the corresponding sexes of the S_1 and S_2 strains. The F_1 rats of either sex are larger than the F_2 rats of the same sex, and while the F_2 rats are slightly more variable than the F_1's the difference is so slight that it is not significant. In comparing the size and variability of the F_2 generation with the parent strains, we find the F_2 rats to be significantly larger than the larger parent strain and less variable

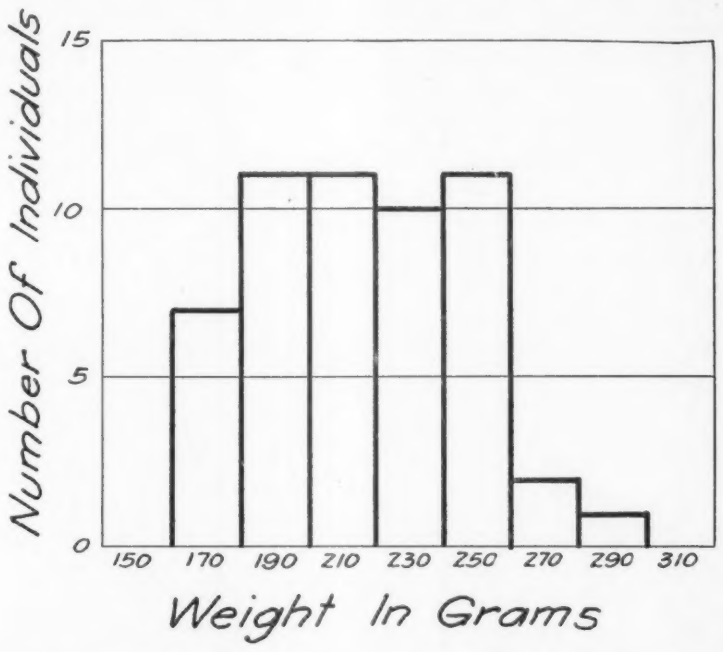

FIGURE 15.—Frequency polygon showing variation in weight of 53 F_1 ($S_1 \times S_3$) males at 90 days of age.

than either parent strain. This difference in variability, however, is not significant in the case of the S_1 and F_2 females.

Table 4 contains the comparative data on the difference in the mean weights and coefficients of variability of groups of rats connected with the S_1 by S_3 cross. These data show that both males and females of the S_3 strain are significantly larger than the males and females of the S_1 strain, and that there is not a large difference in the variability of the two

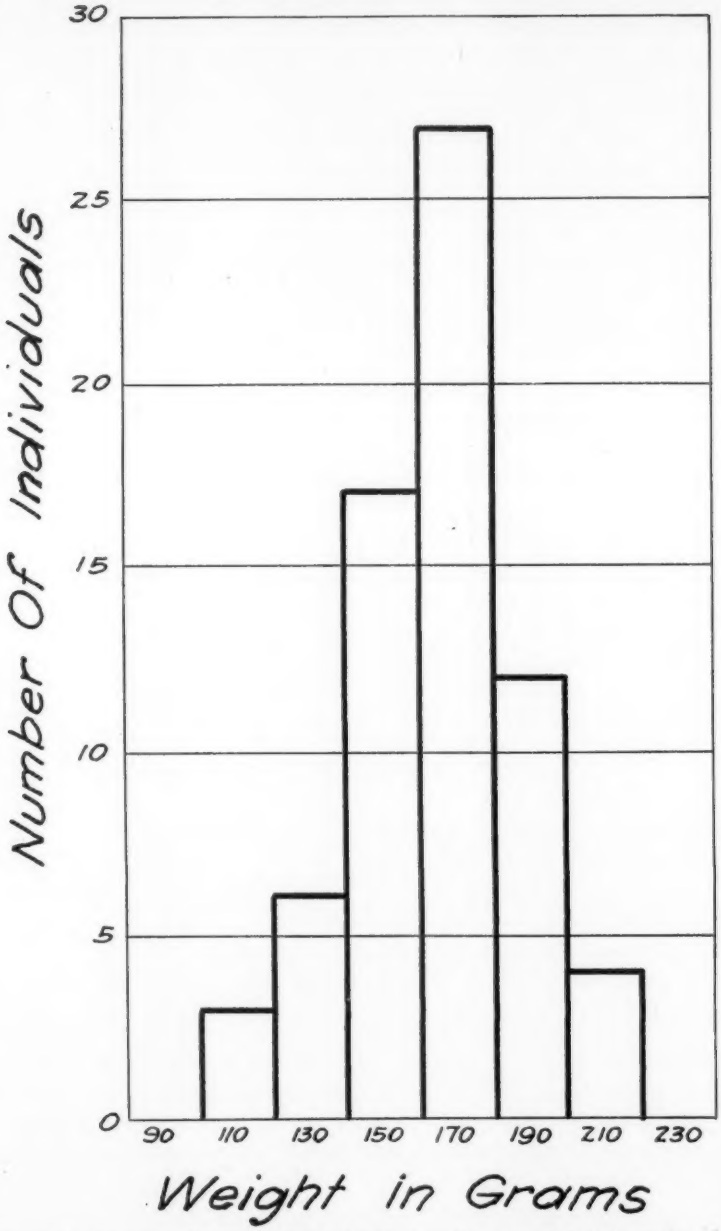

FIGURE 16.—Frequency polygon showing variation in weight of 69 $F_1(S_1 \times S_3)$ females at 90 days of age.

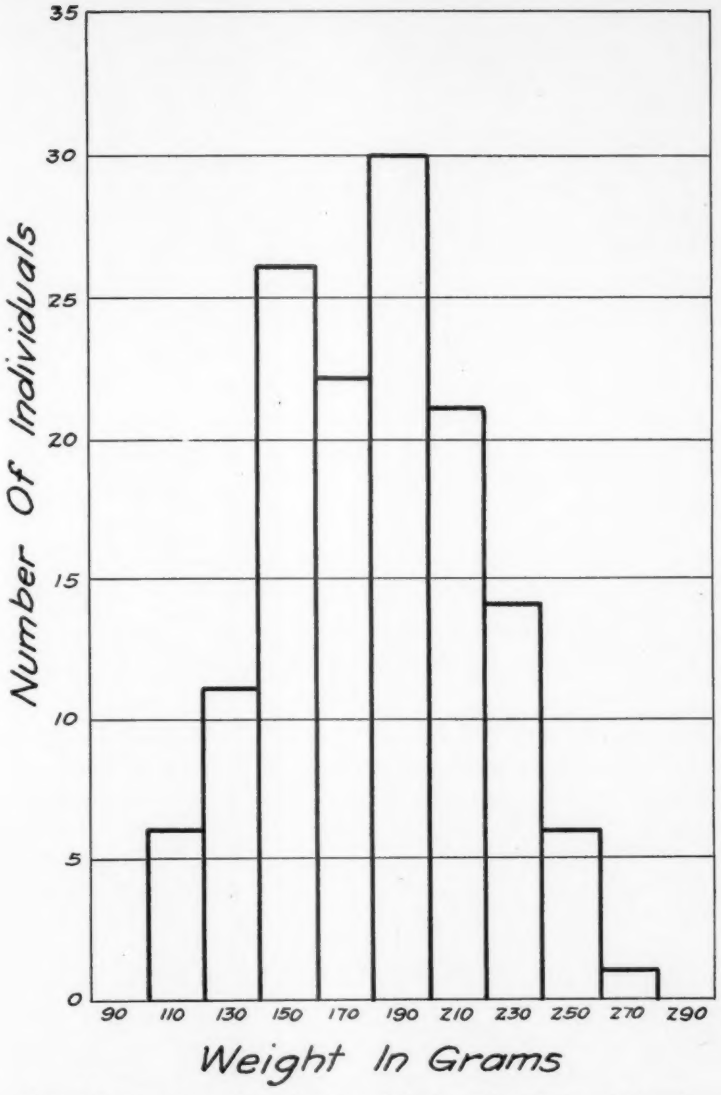

FIGURE 17.—Frequency polygon showing variation in weight of 137 F_2 ($S_1 \times S_3$) males at 90 days of age.

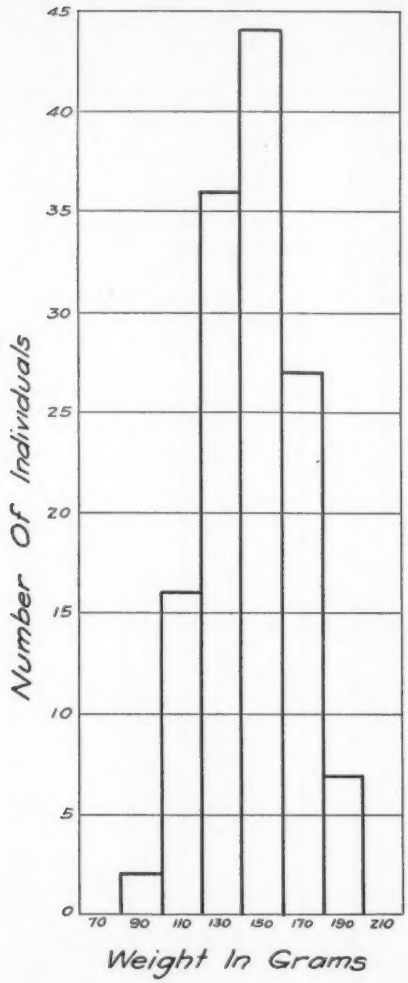

FIGURE 18.—Frequency polygon showing variation in weight of 132 $F_2(S_1 \times S_9)$ females at 90 days of age.

TABLE 2

Statistical study of weights of rats of strains S_1, S_2, and S_3, and of their F_1 and F_2 progeny at age 90 days.

STOCK	SEX	NO	*M*	*S*	C. OF V.
S_1	Males	100	173.0±3.17	47.02±2.24	27.17±1.29
S_1	Females	98	137.8±1.64	24.03±1.16	17.43±0.84
S_2	Males	52	131.9±3.18	34.01±2.24	25.78±1.70
S_2	Females	59	104.3±2.14	24.43±1.51	23.43±1.45
S_3	Males	62	203.9±3.98	44.80±2.70	21.95±1.33
S_3	Females	80	156.3±2.52	34.44±1.77	21.30±1.13
$F_1(S_1 \times S_2)$	Males	48	209.2±2.48	25.47±1.75	12.12±0.83
$F_1(S_1 \times S_2)$	Females	37	159.2±2.55	23.06±1.81	14.48±1.13
$F_1(S_1 \times S_3)$	Males	53	218.4±2.85	29.95±2.02	13.71±0.95
$F_1(S_1 \times S_3)$	Females	69	164.3±1.65	19.49±1.16	11.86±0.71
$F_2(S_1 \times S_2)$	Males	51	186.9±2.40	28.39±1.69	15.18±1.01
$F_2(S_1 \times S_2)$	Females	64	145.0±1.83	21.79±1.30	15.02±0.89
$F_2(S_1 \times S_3)$	Males	137	181.3±2.02	35.01±1.42	19.31±0.79
$F_2(S_1 \times S_3)$	Females	132	145.0±1.31	22.34±0.93	15.40±0.64

The mean weights are slightly larger in this table than the average weights at 90 days of age as shown in table 1. The value of frequencies was made slightly higher by uniformly throwing all weights which fell on group weight divisions into the higher groups.

strains of rats. In comparing the stock strains, S_1 and S_3, with their F_1 progeny we find that the F_1 rats are larger than either parent strain. However, the difference in size is not large enough in the case of the S_3 and F_1 females to indicate a positive difference in size. In comparing the F_1 and F_2 rats we find that the rats of the F_1 generation are larger and less variable than the F_2's. In comparing stock rats with the F_2 generation, the data show that the F_2 rats are intermediate in size between the two stock strains and less variable than either of the stock strains.

Known Genes not Linked with Growth Genes

Since there is a higher coefficient of variability with respect to weight among the F_2 rats than among the F_1 rats of the $S_1 \times S_3$ cross, it should be of interest to group the F_2 rats in a manner which might show whether there is any major growth gene linked with the known genes studied. It should be kept in mind that only three pairs of chromosomes are involved in this study.

TABLE 3

Comparison of means and coefficients of variability in S_1 and S_2 stocks and in their F_1 and F_2 progeny.

	DIFFERENCE IN MEAN WEIGHT	LARGER	DIFFERENCE IN C. OF V.	MORE VARIABLE
S_1 and S_2 Males	41.1±4.48	S_1	1.38±2.13	S_1 (N.S.)
S_1 and S_2 Females	33.5±2.69	S_1	6.00±1.66	S_2
S_1 and F_1 Males	36.2±4.02	F_1	15.05±1.53	S_1
S_1 and F_1 Females	21.4±3.02	F_1	15.27±1.81	S_1
S_2 and F_1 Males	77.3±4.03	F_1	13.67±1.89	S_2
S_2 and F_1 Females	54.9±3.32	F_1	8.95±1.83	S_2
F_1 and F_2 Males	22.3±3.45	F_1	3.06±1.30	F_2 (N.S.)
F_1 and F_2 Females	14.2±3.13	F_1	0.54±1.43	F_2 (N.S.)
S_1 and F_2 Males	13.9±3.97	F_2	11.99±1.63	S_1
S_1 and F_2 Females	7.2±2.45	F_2	2.41±1.22	S_1 (N.S.)
S_2 and F_2 Males	55.0±3.98	F_2	10.61±1.97	S_2
S_2 and F_2 Females	40.7±2.81	F_2	8.41±1.70	S_2

N. S. as used in tables 3 and 4 indicates "not significant."

TABLE 4

Comparison of means and coefficients of variability in S_1 and S_3 stocks and in their F_1 and F_2 progeny.

	DIFFERENCE IN MEAN WEIGHT	LARGER	DIFFERENCE IN C. OF V.	MORE VARIABLE
S_1 and S_3 Males	30.9±5.09	S_3	5.22±1.79	S_1
S_1 and S_3 Females	18.5±3.00	S_3	3.96±1.40	S_3 (N.S.)
S_1 and F_1 Males	45.4±4.25	F_1	13.46±1.60	S_1
S_1 and F_1 Females	26.5±2.32	F_1	5.57±1.09	S_1
S_3 and F_1 Males	14.5±4.98	F_1	8.24±1.62	S_3
S_3 and F_1 Females	8.0±3.00	F_1 (N.S.)	9.53±1.33	S_3
F_1 and F_2 Males	37.1±3.48	F_1	5.60±1.23	F_2
F_1 and F_2 Females	19.3±2.10	F_1	3.54±0.99	F_2
S_1 and F_2 Males	8.3±3.75	F_2 (N.S.)	7.86±1.50	S_1
S_1 and F_2 Females	7.2±2.09	F_2	2.03±1.04	S_1 (N.S.)
S_3 and F_2 Males	22.6±4.45	S_3	2.64±1.54	S_3 (N.S.)
S_3 and F_2 Females	11.3±2.83	S_3	5.99±1.32	S_3

In crossing the S_1 (*CCppRRHHAA*) and S_2 (*ccPPRRhhaa*) stock strains we obtain F_1 rats which are agouti in color and of the genetic constitution *CcHhAa*. In the F_2 generation we get all the possible combinations of these three allelomorphic pairs of genes; therefore, if there are major growth genes carried on the same chromosomes, they should be brought to light by the F_2 groupings made in table 5. The data of table 5 do not give any definite evidence that growth genes are linked with the known

TABLE 5

Statistical study of weights of F_2 animals, $S_1 \times S_2$ cross, at 90 days of age.

SEX	GENES	NUMBER OF RATS	*M*	*S. D.*	C. OF V.
Males	*CC*	45	185.1±3.11	31.02±2.20	16.97±1.20
"	*Cc*	60	181.3±3.24	37.29±2.29	15.05±0.92
"	*cc*	36	169.5±3.87	34.47±2.74	20.33±1.61
"	*HH*	51	168.5±3.05	32.38±2.16	19.21±1.28
"	*Hh*	62	190.7±3.27	38.24±2.32	20.05±1.21
"	*hh*	28	177.9±3.75	29.46±2.65	16.43±1.48
"	*AA*	43	186.7±3.65	35.55±2.58	19.03±1.38
"	*Aa*	64	177.8±2.86	34.01±2.02	19.12±1.14
"	*aa*	33	172.4±4.76	40.52±3.36	23.50±1.95
Females	*CC*	27	138.2±3.25	25.12±2.30	18.17±1.66
"	*Cc*	72	143.9±1.67	21.00±1.18	14.58±0.81
"	*cc*	34	147.6±2.24	19.39±1.58	13.12±1.06
"	*HH*	33	145.6±2.32	20.09±1.64	13.79±1.12
"	*Hh*	67	141.4±1.87	22.74±1.32	16.08±0.93
"	*hh*	33	145.1±2.65	22.58±1.87	15.15±1.29
"	*AA*	29	140.4±2.62	20.78±1.84	14.80±1.31
"	*Aa*	74	144.9±1.30	23.02±1.27	15.88±0.88
"	*aa*	30	142.7±2.62	21.60±1.88	15.13±1.31

The data of table 5 are based on the weights of seven more rats than the number used in previous tables. The data on the rats which were added at this point were not complete when previous tables were compiled.

genes. In the case of the F_2 males there is an indication that growth genes are carried on the same chromosome with the gene (*C*) for chromogen. Male rats homozygous for this gene are slightly larger than those in a heterozygous condition, and those in a heterozygous condition are larger than pure recessives (those homozygous for the gene *c* for albinism). This condition does not hold true for the females and therefore cannot be true for the males, unless there is a complementary action between growth

TABLE 6

F_2 Males ($S_1 \times S_3$) weighing 225 grams or more at 90 days of age.

NUMBER	PHENOTYPE	GENOTYPE	NUMBER IN LITTER	AGE IN DAYS				
				30	50	70	90	150
20c	agouti	*Cc Hh Aa*	5 (1)	65	135	220	275	340
40a	black	*Cc Hh aa*	10 (3)	50	100	175	255	320
55a	black hooded	*Cc hh aa*	6	55	100	170	255	330
21c	agouti	Failed to breed	5 (1)	65	145	210	250	330
1a	p. e. yellow	*Cc Hh AA*	8 (2)	65	110	175	245	330
10a	p. e. yellow	*CC Hh AA*	8	65	110	165	240	320
14c	p. e. yellow	*CC Hh Aa*	10	50	110	180	240	330
4a	albino	*cc HH Aa*	8 (2)	65	105	170	235	335
39a	agouti	*Cc Hh AA*	10 (3)	50	100	160	235	300
71a	albino	*cc Hh AA*	8 (5)	40	110	165	230	300
88b	albino	*cc Hh Aa*	8	50	120	180	230	270
92b	black	*Cc Hh aa*	9	50	135	185	230	280
27a	p. e. yellow	*Cc HH Aa*	8 (4)	65	120	175	225	280
28a	p. e. cream	*CC Hh aa*	8 (4)	65	115	170	225	245
73a	agouti	*Cc Hh Aa*	8 (5)	40	100	160	225	280
11c	agouti	*CC Hh AA*	10 (6)	40	105	175	225	290
12c	agouti	*CC HH AA*	10 (6)	50	125	180	225	300

F_2 Males ($S_1 \times S_3$) weighing 135 grams or less at 90 days of age.

80a	black	*Cc HH AA*	8 (1)	30	60	100	135	240
81a	p. e. yellow	*CC HH Aa*	8 (1)	30	60	100	130	195
82a	p. e. yellow	*CC HH AA*	8 (1)	30	55	100	130	230
90a	albino	*cc hh aa*	9	35	65	95	130	220
56b	albino	*cc hh aa*	8	30	55	85	130	205
50c	albino	*cc Hh Aa*	7	45	70	110	130	190
70c	agouti	*Cc HH Aa*	8 (2)	40	80	110	130	175
12b	albino	*cc Hh Aa*	8	35	50	80	125	200
86c	albino	*cc Hh Aa*	9	20	45	75	125	180
4b	agouti	*Cc Hh Aa*	10 (3)	35	50	90	120	180
27b	agouti	*Cc HH Aa*	11	25	45	70	120	200
71c	agouti	*CC HH Aa*	8 (2)	25	60	110	120	180
8b	p. e. yellow	*Cc Hh AA*	10 (3)	30	40	80	120	200
3b	agouti	*Cc Hh Aa*	10 (3)	30	50	85	115	190
62b	black	*Cc Hh aa*	9	20	55	70	115	175
10b	albino	*cc HH AA*	10 (3)	30	40	70	110	180
79a	albino	*cc Hh aa*	8 (1)	30	45	75	105	210
1b	agouti	*Cc HH Aa*	10 (3)	30	50	70	100	195
41b	albino	*cc HH aa*	7	20	40	65	100	190

TABLE 7

F_2 Females ($S_1 \times S_2$) weighing 170 grams or more at 90 days of age.

NUMBER	PHENOTYPE	GENOTYPE	NUMBER IN LITTER	AGE IN DAYS				
				30	50	70	90	150
58a	p. e. yellow	*CC HH Aa*	6 (1)	60	110	160	195	230
16a	agouti	*Cc Hh Aa*	8 (3)	60	105	155	190	190
72b	agouti	*Cc Hh Aa*	8	30	90	125	180	230
42a	albino	*cc Hh Aa*	10 (2)	45	85	140	180	210
8a	albino	*cc HH Aa*	8	55	100	155	180	220
83b	agouti hooded	*Cc hh Aa*	5	65	130	160	180	205
57a	albino	*cc hh aa*	6 (1)	50	105	150	175	215
43a	albino	*cc hh aa*	10 (2)	50	90	135	175	210
66a	agouti	*Cc Hh Aa*	10	45	95	140	170	200
8c	agouti hooded	*Cc hh Aa*	8	50	100	145	170	195
51a	albino	*cc Hh Aa*	9	45	90	130	170	200
14a	black	*Cc Hh aa*	8 (3)	60	100	135	170	210
60a	agouti	*Cc Hh AA*	6 (1)	55	100	140	170	220
89b	agouti hooded	*Cc hh AA*	8	50	105	150	170	200

F_2 Females ($S_1 \times S_3$) weighing 115 grams or less at 90 days of age.

51b	agouti hooded	*Cc hh Aa*	10	30	60	90	115	170
44c	p. e. yellow	*CC HH Aa*	6	40	75	100	115	140
92a	black	*Cc Hh aa*	9 (1)	30	60	95	115	200
5b	black	*Cc HH aa*	10 (2)	35	55	95	115	135
7b	agouti	*Cc Hh AA*	10 (2)	35	55	100	115	130
43b	p. e. yellow hooded	*CC hh AA*	7	20	50	80	110	160
16b	agouti	*Cc Hh Aa*	8 (3)	30	50	75	110	160
15b	agouti	*Cc Hh Aa*	8 (3)	40	55	80	110	140
95a	albino	*cc Hh aa*	9 (1)	30	50	80	110	160
93a	agouti	*Cc Hh AA*	9 (1)	30	60	85	110	155
18b	p. e. hooded yellow	*CC hh Aa*	8 (3)	40	60	80	110	150
77b	p. e. hooded yellow	*CC hh Aa*	8	25	50	90	110	140
79c	agouti	*Cc hh Aa*	9 (5)	25	50	75	105	105
86a	albino	*cc HH aa*	8 (4)	25	50	70	100	190
85a	p. e. yellow	*CC Hh Aa*	8 (4)	30	50	75	100	165
14b	agouti	*Cc Hh AA*	8 (3)	40	60	80	100	140
81c	p. e. hooded yellow	*Cc hh Aa*	9 (5)	25	45	70	100	145
83a	black	*CC Hh aa*	8 (4)	30	45	70	90	160
84a	agouti	*CC Hh AA*	8 (4)	30	40	60	85	150

genes linked with the gene for chromogen and genes carried on the Y chromosome. The same condition exists in the case of males of the agouti (*A*) and non-agouti (*a*) gene group, but again the females of the same gene grouping fail to show any indication of growth genes being carried on the same chromosome with the agouti gene.

Grouping of the Heavy Weight and Light Weight rats

The data of table 5 do not throw any definite light upon the cause of the greater variability in weight of F_2 rats when compared to F_1 rats, and it will be of interest to group the heavy and light F_2 rats of each sex and study their genetic constitution. Tables 6 and 7 contain such groups and in connection with this grouping complete data on each rat are given.

At this point it might be well to indicate the significance of the small letters following the number of each rat and also the numbers in parenthesis following the number of rats in the litter to which these rats belong. The system used in ear marking the rats did not extend beyond ninety-nine; therefore, the numbers of the first ninety-nine F_2 rats are followed by a small "a," the second ninety-nine by a small "b" and the third by by a small "c" to distinguish whether they belong to the first, second or third group. The numbers in parenthesis indicate that more than one rat in the male or female division of the table belongs to the same litter, and this same number is placed after each male or female coming from one litter.

Table 6 includes the F_2 male rats weighing 225 grams or more at 90 days of age and the F_2 male rats weighing 135 grams or less at 90 days of age. This table shows that among 141 male rats there were 17 falling in the heavy group and 19 falling in the light group. The 17 heavy weight rats came from 11 different litters and the size of the litters from which they came varied from five to ten young per litter. The 19 light weight rats came from 11 different litters and the size of the litters from which they came varied from six to ten young per litter.

The weights of nine rats (numbers 1b, 3b, 4b, 8b, 10b, 79a, 80a, 81a, and 82a) in the light group can be explained on a nutritional basis and should not be considered from the standpoint of heredity. Rats bearing numbers 1b, 3b, 4b, 8b, and 10b are litter mates from a litter of ten in number. This litter was marked at weaning time as being in an unthrifty condition. This unthrifty condition was indicated by rough coats and lack of vitality. There were six male rats and four females in the litter. One male died when fifty-five days of age and the remaining five males all fall into the light group of males. Two of the four females of this litter

(numbers 5b and 7b) are to be found in the light group of females, table 7. Rats bearing numbers 79a, 80a, 81a, and 82a are litter mates and from a litter of eight in number. This litter was noted at weaning time as being in the same physical condition as the litter previously discussed. There were four males and four females in this litter. All males are found in the light group and all their litter sisters (numbered 83a, 84a, 85a, and 86a) are found in the light group of females (table 7). These are the only rats included in tables 6 and 7 of which a poor physical condition was observed during the time they were on the experiment. By eliminating the nine rats of these two litters there remain ten male rats in the light weight group and these ten rats represent nine different litters.

In studying the remaining rats of these two groups, there is no evidence of any linkage between the known genes and growth genes. Their known genetic combinations are as varied as could be expected in so small a number. They come from a relatively large number of litters and from litters of large size as well as from litters of medium size. There is no evidence that the rats of the heavy group are from smaller sized litters than are the rats of the light group. In fact by the elimination of only one of the litters as previously discussed there would not remain a single rat in the light group coming from a litter above nine in number, while there are five rats in the heavy group belonging to three different litters of ten in number. This would indicate that size is probably inherited as a multiple gene condition, and that chromosomes other than those studied carry the major growth genes, if such exist.

The diploid number of chromosomes of the rat (*Rattus Norvegicus*) according to the recent work of PINCUS (1927) is 42. He found an unequal pair in the spermatogonial divisions which he concluded were the sex chromosomes. Since the present study involves only six diploid chromosomes there is still an ample number of chromosomes not included in the study for an assumption that there are size genes carried on the unstudied chromosomes.

Table 7, which has been referred to in my discussion of table 6, includes the F_2 female rats weighing 170 grams or more at 90 days of age and the F_2 female rats weighing 115 grams or less at 90 days of age. This table shows that among 133 female rats there were 14 falling in the heavy weight group and 19 falling in the light weight group. The 14 heavy weight rats came from 10 different litters and the size of the litters from which they came varied from five to ten young per litter. The 19 light weight rats came from 9 different litters and the size of the litters from which they came varied from six to ten per litter. If we eliminate rats

of the light group belonging to the two litters discussed in connection with table 6, there will remain 13 rats in the light weight group. These 13 rats came from seven different litters and the size of the litters from which they came varied from six to ten young per litter.

The same conclusions can be drawn from the data of table 7 as were drawn from the data of table 6. There is no evidence that size genes are carried on the three pairs of chromosomes studied and no evidence that the size of the litters from which the two groups of rats came influenced the

TABLE 8

Variation in litter size and weight and correlation between litter size and weight of 141 F_2 ($S_1 \times S_3$) males at 90 days of age.

	SIZE OF LITTER										
WEIGHT IN GRAMS	3	4	5	6	7	8	9	10	11	12	TOTALS
100–105					1	1		1			3
110–115							1	2			3
120–125						2	1	2	1		6
130–135					1	5	1				7
140–145					1	4	2	3	3		13
150–155		1		3	3	1	4	2			14
160–165		1		1	4	3	1	1			11
170–175					2	2	5	2			11
180–185	1				4	7	1	5			18
190–195					2	6	3	2			13
200–205	1		1			2	6	3			13
210–215			2		1	3	1	2			9
220–225				1		3	2	3			9
230–235						3	1	1			5
240–245						2		1			3
250–255				1				1			2
260–265											
270–275			1								1
TOTALS	2	2	4	6	19	44	29	31	4		141

Mean litter size = 8.30 — Mean weight = 177.2 grams
Standard deviation = 1.54 — Standard deviation = 35.54 grams
Coefficient of variation = 18.55 percent — Coefficient of variation = 20.05 percent
Coefficient of correlation (litter size and weight) = 0.10 ± 0.05

size of the rats at ninety days of age. The only explanation which we can offer as to the size difference of these two groups of F_2 rats is that size is inherited as a multiple gene condition and that the major growth genes are carried on the unstudied chromosomes.

Correlation Between Litter Size and Weight

Tables 6 and 7 indicate that size of litter has little if any effect upon the weight of F_2 ($S_1 \times S_2$) rats. In order to know the definite relationship between litter size and weight, correlation tables have been made for both male and female rats. Tables 8 and 9 show that a very slight correlation,

TABLE 9

Variation in litter size and weight and correlation between litter size and weight of 133 F_2 ($S_1 \times S_3$) females at 90 days of age.

WEIGHT IN GRAMS	SIZE OF LITTER 3	4	5	6	7	8	9	10	11	TOTALS
80–85						1				1
90–95						1				1
100–105						3	2			5
110–115				1	1	3	4	3		12
120–125				1	3	5	3	4		16
130–135		1			3	5	4	6	5	24
140–145		1	2	2	3	6	3	2	2	21
150–155			1	1	1	6	7	5	1	22
160–165					3	7	3	3		16
170–175			1	2		3	1	2		9
180–185			1			2		1		4
190–195				1		1				2
TOTALS:		2	5	8	14	43	27	26	8	133

Mean litter size = 8.38 — Mean weight = 141.30 grams
Standard deviation = 1.52 — Standard deviation = 21.52 grams
Coefficient of variation = 18.13 percent — Coefficient of variation = 15.23 percent
Coefficient of correlation (litter size and weight) = 0.12 ± 0.06

but of doubtful significance, exists between the litter size and weight of F_2 rats at 90 days of age, the coefficient of correlation being 0.10 ± 0.05 for the male rats and 0.12 ± 0.06 for the females. These coefficients of correlations indicate that we are safe in saying that the size of the litter had very little effect upon the weights of the rats of the F_2 generation. These tables include the two litters which were eliminated from tables 6 and 7 on the ground that their small size could not be due to heredity.

Various Gene Combinations

Table 10 gives the average growth weights of $F_2(S_1 \times S_3)$ rats of various gene combinations. This table included all tested F_2 rats, or 141 males and 133 females. The rats were first grouped according to the number of

dominant genes and then according to the homozygous or heterozygous condition of the genes. After a careful study of the data of this table,

TABLE 10

Average weights of $F_2(S_1 \times S_3)$ animals of various gene combinations at ages 30–150 days.

SEX	GENE COMBINATION	NUMBER OF INDIVIDUALS	AGE IN DAYS				
			30	50	70	90	150
Males	3 Dominant	67	42.98	88.95	134.32	180.37	266.41
"	2 "	55	40.90	85.72	131.45	175.09	241.27
"	1 "	15	44.33	84.33	128.33	176.66	242.00
"	0 "	4	32.50	68.75	108.75	150.00	222.50
Females	3 "	59	40.00	80.33	141.61	139.70	176.77
"	2 "	55	40.54	78.81	112.00	139.40	177.72
"	1 "	15	40.33	82.66	113.00	141.66	182.33
"	0 "	4	43.75	91.25	135.00	161.25	197.50
Males	3 Homozygous	26	39.61	80.38	117.69	165.60	232.69
"	2 "	56	43.05	89.28	134.10	179.00	248.30
"	1 "	47	42.97	88.29	135.51	179.20	243.51
"	3 Heterozygous	12	38.75	81.25	132.08	180.40	241.66
Females	3 Homozygous	16	39.06	82.18	117.50	142.80	183.75
"	2 "	46	42.93	82.60	114.67	139.90	180.65
"	1 "	46	40.10	78.15	109.13	140.10	179.02
"	3 Heterozygous	25	38.20	78.80	110.80	140.20	176.80

I am convinced that there is no relation between the size of rats and the number of known dominant genes, or between size and the homozygous and the heterozygous condition of the genes studied.

CONCLUSIONS ON EXPERIMENTAL WORK

In this study of three known pairs of allelomorphic genes there is no evidence that the known dominant genes, or that the heterozygous condition of the genes studied has any influence on the size of F_2 rats of the S_1 and S_3 cross.

There is no definite evidence that growth genes are linked with the genes studied, but on the other hand there is no evidence that growth genes are not carried on chromosomes not studied. The rats of the F_2 generation of the S_1 by S_3 cross were more variable than the F_1 rats of this cross, and when the heavy and light rats of the F_2 generation are grouped for study there is evidence that the difference in size of the rats of these two groups

must be due to hereditary size genes probably carried on the unstudied chromosomes.

This study does not give an answer to the question as to the cause of hybrid vigor or heterosis, but it does give additional information on the three pairs of chromosomes studied.

SUMMARY OF EXPERIMENTAL RESULTS

1. Hybrid vigor or heterosis was shown by the F_1 rats in each cross studied.

2. Heterosis was more marked in the F_1 rats of the S_1 by S_2 cross than in the F_1 rats of the S_1 by S_3 cross, as would be expected since there was a greater difference in the size of the parent races in the former cross.

3. There was a distinct loss of vigor shown by the F_2 rats of each cross as compared to the F_1 rats.

4. This loss of vigor was more marked in the case of the F_2 rats of the S_1 by S_3 cross.

5. The F_1 rats of each cross were less variable in weight than the rats of the respective parent strains.

6. The F_2 rats of each cross were more variable in weight than F_1 rats, although the difference in the variability was not sufficient to be significant in the case of the F_2 rats of the S_1 by S_2 cross.

7. The F_2 rats of each cross were less variable than the rats of the respective parent strains.

8. The grouping of F_2 rats of the S_1 by S_3 cross according to known allelomorphic genes does not show any definite evidence that the three pair of chromosomes studied carry growth genes.

9. The grouping of the heavy weight and light weight F_2 rats does not show any relation between the size of the rats and their known genetic constitution. This grouping also fails to indicate that size of the litters, from which the heavy weight and light weight rats came, has influenced the weight of the F_2 rats.

10. There exists a very slight correlation between the litter size and the weight of F_2 rats, but this correlation is too small to account for the extreme difference in the weights of rats contained in the heavy weight and the light weight groups of tables 6 and 7.

11. The grouping of F_2 rats according to the number of known dominant genes in their genetic constitution does not show any evidence that these genes influence the size of the rats.

12. The grouping of F_2 rats according to the homozygous or heterozygous condition of the known allelomorphic genes does not show any evi-

dence that either the homozygous or heterozygous state has any influence on the size of the rats.

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THE CYTOLOGY AND GENETICS OF A HAPLOID SPORT FROM *OENOTHERA FRANCISCANA*[1]

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The first haploid spermatophyte to be recorded was from *Datura stramonium* (BLAKESLEE and others 1922), and later studies have shown them to be not infrequent sports from this species apparently appearing without marked relation to the temperature of the season. They have also been obtained by the application of pollen from *Datura ferox* to the stigma of *D. stramonium*. BELLING and BLAKESLEE (1923, 1927) presented a cytological study of these plants. Shortly after, CLAUSEN and MANN (1924) described two haploid plants from the F_1 of the cross *Nicotiana tabacum* $\times$ *N. sylvestris*, and later CHIPMAN and GOODSPEED (1927) gave an account of the haploid from *Nicotiana tabacum* var. *purpurea*. RUTTLE (1928) has reported three additional haploids from *tabacum* var. *purpurea* and two new plants from *tabacum* var. *Cuba*. GAINES and AASE (1927) in attempts to pollinate a hybrid of Triticum from Aegilops obtained a haploid wheat plant and described its cytology. LESLEY and FROST (1928) found a dwarf plant of Mattiola having the haploid set of normal chromosomes and in addition a chromosome fragment. HOLLINGSHEAD (1928) records two haploids of *Crepis capillaris* from the F_1 of the cross *capillaris* $\times$ *tectorum*. JÖRGENSEN (1928) has obtained a number of haploids of *Solanum nigrum* and *S. nigrum gracile* through capsules stimulated to parthenocarpic development by pollinations from *S. luteum* and other forms that hybridize only rarely with *S. nigrum*. JÖRGENSEN gives an account of the cytology of these Solanum haploids in which the behavior at meiosis is very different from that in Triticum, Nicotiana, Datura and as we shall see in Oenothera. LINDSTROM (1929) described a haploid from tomato almost completely pollen sterile but producing a few seeds after pollinations from other varieties. Our paper adds to the list the first haploid Oenothera but it seems probable that other plants of this character will shortly be recognized in this genus.

The possibility of apomixis in Oenothera has been strongly indicated by the interesting results of HABERLANDT (1921, 1922, 1927) in connection

[1] Cytological Studies on Oenothera IV. Papers from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 303.

with his experiments on the stimulus to growth resulting from wounds. By pinching the ovaries, by pricking them with a needle, and by the castration of young flowers he obtained early stages of parthenogenetic embryos and adventitious embryos in *Oenothera Lamarckiana*, together with endosperm development. HABERLANDT holds that the exciting agents are hormones liberated as the result of the wounding of tissue or the death of cells. Similar developments were noted in ovaries of *Lamarckiana* and *muricata* at the end of the flowering season and are believed by him to result from products formed by the aging plants.

There have been other suggestions of possible apomixis in Oenothera. LUTZ (1909) reported two plants of *lata* (15 chromosomes) in the F_1 of the cross *lata*×*gigas*. GATES (1909, 1924) performed experiments on *Lamarckiana-lata*, *biennis-lata* and *gigas* but obtained no seed. The brief statement of HAIG-THOMAS (1913) of parthenogenesis in *Oneothera biennis* following the castration of young flower buds can scarcely be accepted as a demonstration. It may happen that parthenogenesis in Oenothera will result, as JÖRGENSEN (1928) found in Solanum, through pollinations from types that will induce parthenocarpy or through a very limited amount of self pollination sufficient to start the development of fruit and with this growth give physiological conditions favorable to forms of apomixis. The well known reactions of many plants as expressed in parthenocarpy show a close physiological relation between the development of fruit and stimuli from pollen tube growth. The maturation of seed in many forms is apparently only possible as it proceeds hand in hand with the development of the fruit.

GENETICS OF THE HAPLOID

Oenothera franciscana Bartlett, text figure 1, is one of the few apparently homozygous forms in this genus of numerous impure or heterozygous species. It is a large vigorous plant with pollen almost wholly perfect, text figure 3A, and with high seed fertility (about 90 percent). As would be expected its chromosomes are characteristically paired at diakinesis and during the first meiotic division. In certain material (CLELAND 1922) a chain of four chromosomes has been found persisting even to metaphase of the heterotypic mitosis but in other material (Kulkarni 1929) this chain breaks into two pairs at diakinesis showing the association to be loose in character. For eight years cultures with a total of 1499 plants gave no exceptions to the type. Then in 1923 appeared the first variant, a sport named *pointed tips* which later was found to be a haploid, presumably from the parthenogenetic development of a *franciscana* egg.

Plants of pointed tips, text-figure 2, are about half the stature of *franciscana* and with all organs proportionally smaller. The leaves are generally narrower and more sharply pointed and the very red bud cones are more attenuate. The flowers are about half the size of the parent species. Pollen is developed only in small amounts and frequently flowers will produce none at all, the anthers being shriveled. Some plants have

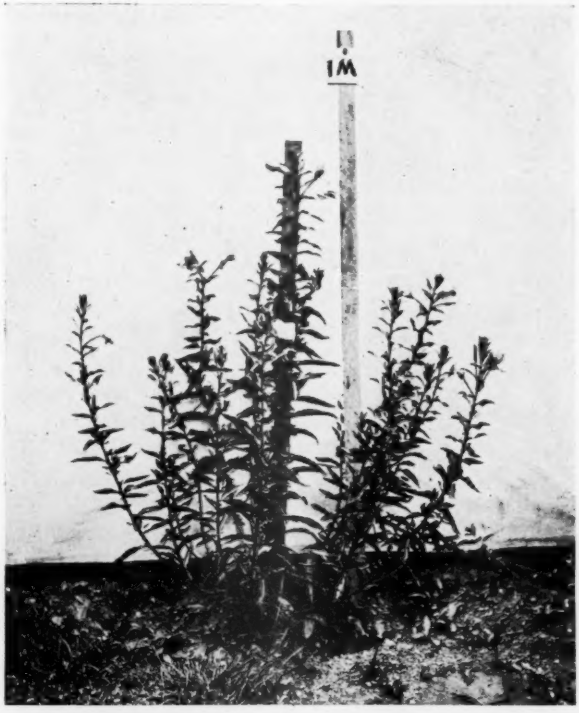

FIGURE 1.—*Oenothera franciscana* early in the flowering season. The central shoot may finally become more than 1 m. high.

been found to develop no pollen over several weeks of daily observation. In such pollen as may be formed, text figure 3B, frequently 60 to 90 percent of the grains will be shrunken. Since the pollen is so highly sterile few seeds are developed on selfed plants and the capsules are thin.

The first plant of pointed tips (23.21-165) was selfed and also backcrossed to the parent line of *franciscana*. The progeny of selfed lines is

almost wholly plants of the parent type, *franciscana*, but a few plants of pointed tips appear. The data of the selfed lines are given in table 1 and it should be noted that cultures 26.25 and 26.26 are second generations and that cultures 27.41, 27.42 and 27.43 are third generations. These selfed lines gave a total of 694 *franciscana* and 29 pointed tips. The behavior of the haploid when selfed was then what would be expected. Its fertile gametes carry the haploid set of *franciscana* chromosomes and their

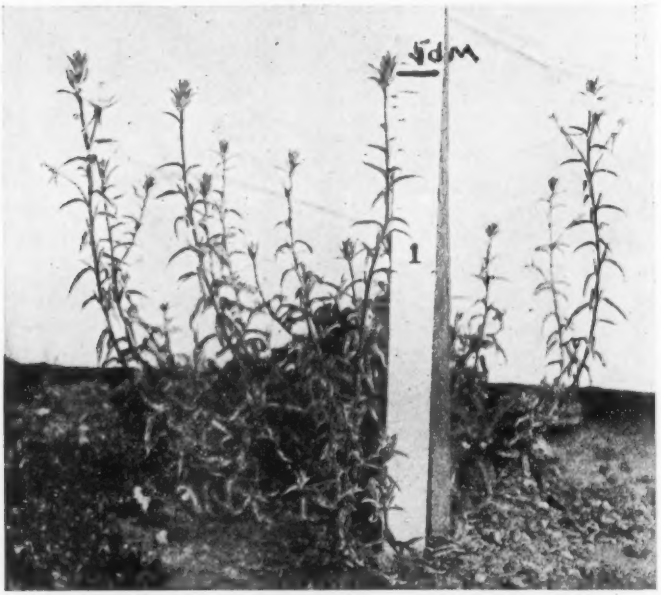

FIGURE 2.—The haploid, pointed tips, a plant of about one half the stature of *Oenothera franciscana*.

fusion would give the diploid zygote of *franciscana*. Such plants of pointed tips as appear are to be considered as coming from eggs that develop parthenogenetically. Of the pointed tips, 22 plants in one line (26.26 and 27.43) presented somewhat broader leaves than the type and a higher degree of pollen fertility; this form has not yet been studied cytologically and may possibly be a modification of the original haploid. A very interesting form, ***red elongate***, which appeared in certain cultures, table I, will not be discussed at this time.

In addition to the first plant of pointed tips (23.21-165) there have appeared from later cultures of *franciscana* 3 more plants:—27.21-1108 from a culture numbering 1117 plants, 28.21-1 from a culture of 945 plants, and 28.61-813 from a culture of 847 plants. The total number of haploids so far thrown by *franciscana* has been 4 plants in thirteen genera-

TABLE 1

Progeny in selfed lines from the first plant of pointed tips (23.21–165).

CULTURE	PARENT POINTED TIPS SELFED	PERCENT OF GERMINATION	SEEDLINGS	FRANCISCANA	POINTED TIPS	RED ELONGATE	FAILED TO MATURE	DIED
24.25 1st generation	23.21-165	94	361	329	24.25–248 24.25–332		1 dwarf similar to pointed tips	29
26.25 2nd generation	24.25–248	74	37	29	26.25–13 26.25–14 26.25–20			5
26.26 2nd generation	24.25–332	68.2	60	55	26.26–40 somewhat broader leaved		2 narrow-leaved rosettes	2
27.41 3d generation	26.25–13	75	138	107	27.41–82 27.41–130	13	1 large thick-leavedrosette 8 narrow-leaved rosettes	7
27.42 3d generation	26.25–14	25.7	51	39		3	1 narrow-leaved rosette	8
27.43 3d generation	26.26–40	53.4	164	135	21 plants somewhat broader leaved	1	1 large thick-leaved rosette	6
Total			811	694	29	17	14	57

tions that have totaled 5690 plants. However, some of these generations have been small and did not express the results of complete seed germination so that the ratio of pointed tips out of *franciscana* is undoubtedly higher than these figures might suggest. Since the cultures 27.21, 28.21, and 28.61 were from seeds forced to complete germination and gave 3

pointed tips in a total of 2909 plants, it seems probable that the ratio of the haploid to *franciscana* is about 1:1000.

Pointed tips has also appeared in some crosses of *franciscana* to certain derivatives upon which the senior author is making studies on the inheritance of the *sulfurea* color and of dwarfness. The following three crosses threw each a single plant of the haploid:—culture 26.29 *franciscana*×*franciscana sulfurea*, 49 plants, gave pointed tips 26.29-6; culture 26.58 *franciscana*×(*franciscana*×*franciscana sulfurea* dwarf), 254 plants, gave pointed tips 26.58-103; culture 26.61 (*franciscana sulfurea* dwarf× *franciscana*)×*franciscana*, 214 plants, gave pointed tips 26.61-84. It is clear that pointed tips in cultures 26.29 and 26.58 might have come from the parthenogenetic development of *franciscana* eggs. Culture 26.61 must be briefly explained: its female parent was the hybrid *franciscana sulfurea* dwarf (*yytt*)×*franciscana* (*YYTT*); *Y* stands for yellow petals and *T* for normal height, absence of color (sulfurea) and dwarfness being recessive. The hybrid has the constitution *YyTt* and forms gametes *YT*, *Yt*, *yT* and *yt*. The egg *YT* has the chromosome set of *franciscana* and its parthenogenetic development would give pointed tips. The progeny of the three plants of pointed tips is given in table 2. As would be

TABLE 2

Progeny from pointed tips out of crosses involving franciscana (f), franciscana sulfurea (fs), and franciscana sulfurea dwarf (fsd).

CULTURE	PARENT POINTED TIPS SELFED	PARENT CROSS	PERCENT OF GERMINATION	SEEDLINGS	FRANCISCANA	POINTED TIPS	OTHER FORMS	FAILED TO MATURE	DIED
27.44	26.29-6	*f*×*fs*	28.7	277	249	none	8 broad-leaved dwarfs 3 thick, broad leaves	6 weak rosettes	11
27.45	26.58-103	*f*×*fsd*	39.2	126	115	27.45-112	2 light green 1 thick, broad-leaved rosette	3 weak rosettes	4
27.46	26.61-84	(*fsd*×*f*)×*f*	45.1	60	53	none	1 broad-leaved dwarf	1 weak rosette 1 narrow-leaved dwarf	4
Total				463	417	1	15	11	19

expected almost all of the plants were *franciscana*. Only one pointed tips appeared, but there were several exceptional plants the most interesting of which were *light green*, a new type in the lineage of *franciscana* and certain large, thick-leaved forms not yet fully studied.

The progeny of the back-crosses of pointed tips to the parent *franciscana*, table 3, was a total of 531 plants of *franciscana* and 3 dwarfs that failed to mature; there were no pointed tips. The *franciscana* plants were to be expected since the haploid gametes of pointed tips when formed

TABLE 3

Backcrosses of the first plant of pointed tips to the parent species franciscana.

CULTURE	BACKCROSS	PERCENT OF GERMINATION	SEEDLINGS	FRANCISCANA	FAILED TO MATURE	DIED
24.26	franciscana×pointed tips 23.21–169×23.21–165	78.9	244	234	2 dwarfs	8
24.27	pointed tips×franciscana 23.21–165×23.21–169	83.7	298	297	1 dwarf	
Total			542	531	3	8

have the same set of chromosomes as that carried by *franciscana* and their union with *franciscana* gametes would give the latter plant. If plants of pointed tips had appeared they would be interpreted as the result of parthenogenetic development of eggs whether from *franciscana* or from pointed tips.

It is appropriate to this paper to record the appearance of pointed tips in a large culture of the so-called *Oenothera Hookeri* of genetical literature. This plant is believed by BARTLETT not to be *Oenothera Hookeri* Torrey and Gray, the type specimen of which has marked canescent pubescence and is perhaps related to *Oenothera venusta* Bartlett. However, for the present it seems best to use the name *Hookeri* for the material first studied by DE VRIES and later by RENNER and others. The material of *Hookeri* is very closely related to *Oenothera franciscana* Bartlett and for purposes of field study might be considered a form of this species. *Hookeri* is not so sturdy a plant but it has the habit, pubescence and bud tips of *franciscana*, differing chiefly in its narrower leaves, bright red stems, red hypanthia, and in having more red on the bud cones. As in *franciscana* the pollen of *Hookeri* is almost wholly perfect and the seed fertility is very high (about 96 percent). *Hookeri* like *franciscana* has pairing chromosomes and is one of the few established homozygous species of Oenothera.

The line of *Hookeri* came from seeds kindly supplied by RENNER. Three generations have been grown:—culture 24.18 matured 202 plants, uniform; culture 27.26 matured 526 plants, uniform except for the plant of pointed tips (27.26-475); cultures 28.26 matured 563 plants, uniform.

Pointed tips from *Hookeri* has then appeared only once in a line of cultures totaling 1291 plants, a frequency similar to that in *franciscana* where the ratio has been about 1:1000. This plant (27.26-475) was typical pointed tips but wholly sterile as observed for 38 days during the flowering season. The flowers over this period produced only shriveled anthers.

In concluding the genetical account of this haploid Oenothera it should be emphasized that in every case the haploid might have arisen through the parthenogenetic development of an egg thus supporting the evidence so far reported for this manner of origin of haploids in plants.

CYTOLOGY OF THE HAPLOID

The material for this cytological study came from the anthers of pointed tips 27.21-1108, the second plant of pointed tips to appear in the direct line of *franciscana*. The best results were from material fixed in the following modifications of Bouin's fluid: Sat. aq. sol. of picric acid 75 cc, commercial formalin 25 cc, glacial acetic acid 5 cc, chromic acid 1 g, urea 2 g. The anthers were left 5 1/2 hours in the fixing fluid, then rinsed in water and run through low grades of alcohol beginning with 2 1/2 percent, thirty minutes in each grade. Strong Flemming also gave satisfactory fixation; weak Flemming proved worthless. Sections were cut about 10μ and stained with iron alum haematoxylin.

The haploid count of 7 chromosomes was obtained in tissues of the developing flower. It is shown in plate 1, figure 1, which is the metaphase of a tapetal cell viewed from the pole.

There will first be described the history, illustrated in figures 2-29, which results in the formation of the very few good pollen grains that carry the normal set of 7 chromosomes. The resting nucleus of the microsporocyte, figure 2, contains a delicate chromatic network with numerous deeply staining bodies distributed over the periphery. As prophase comes on, figure 3, there takes place a gradual shortening and condensation of these strands which later, figure 4, give a coarse reticulum with threads of uneven thickness. The threads of this haploid reticulum are distinctly wider than the threads of the diploid *franciscana* at corresponding stages as shown in the excellent figures of CLELAND (1922, figures 1-4).

The stage of synizesis is clearly marked. It begins with the usual apparent contraction of the reticulum, figure 5, the threads showing irregular thickening. The point of greatest condensation with the contracted reticulum against the flattened nucleolus, figure 6, gives a picture similar to that of *franciscana* except that the threads are not so delicate and the

thread system seems to be not so long (see CLELAND 1922, figures 5-8). The emergence from synizesis, figure 7, through the apparent expansion of loops also follows the usual history in Oenotherae.

Out of synizesis comes the stage of the open spireme, figures 8 and 9, with the thickened threads rather evenly distributed through the nucleus. It becomes evident that the thread system is not so extensive as that in the diploid nucleus of *franciscana* at the corresponding stage. This is shown by a comparison of figures 8 and 9 with figures 14 and 15 given by CLELAND (1922) for *franciscana*.

The stage of second contraction is as clearly marked as in *franciscana*. The chromatic threads thicken and gather towards the center of the nucleus, figure 10. At this time it is evident that regions of the spireme are to become chromosomes, figure 11, although these structures are very closely grouped. With the loosening of the contracted chromatic mass, figures 12 and 13, the 7 chromosomes of this haploid set appear clearly outlined and the period of the second contraction comes to an end.

Then follows the stage corresponding to the characteristic period of diakinesis in diploid plants, but as would be expected there is here no pairing of chromosomes. The 7 chromosomes become irregularly distributed through the nuclear area as shown in figures 14–18. Occasionally two chromosomes may be found attached end to end by a delicate thread, plate II, figures 14 and 15, which possibly represents a portion of the original thread system still persisting.

The nucleus now makes an attempt to carry through a division at this period of the heterotypic mitosis in diploid plants. A multipolar spindle is shown in figure 19, but this fails to pass into the normal bipolar structure in the material which we are now considering. One pole may develop more or less clearly, figures 20 and 21, but the second pole is not formed, the spindle fibers ending over a broad and vague area. The 7 chromosomes move somewhat towards the single pole and then gather in a group and a nuclear membrane is formed around them, figure 22. This gives a nucleus in the period corresponding to interkinesis of normal meiosis but there has been no segregation of chromosomes, no heterotypic mitosis.

At this time the chromosomes divide lengthwise, and the nucleus then contains 7 split chromosomes, figure 23, such as are present at interkinesis in *franciscana*. As in *franciscana* the split does not appear until rather late in this period that corresponds to interkinesis.

Then comes the homoeotypic mitosis which is to separate the halves of the split chromosomes. Its metaphase, figure 24, with the 7 pairs of daughter chromosomes, now much condensed, is similar to the homoeo-

Explanation of Plates

All figures were drawn with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 mm (num. aper. 1.30) in combination with the compensating ocular K 20×. The approximate magnification for figures 1–29 is 2400 diam.; for figures 30–37 it is 1800 diam.

Figures 1–29 give the history which results in the formation of a small proportion of the pollen grains with a normal set of seven chromosomes.

Plate 1

Figure 1.—Metaphase of the mitosis in a tapetal cell as seen from the pole of the spindle, 7 chromosomes.

Figure 2.—Resting nucleus of the microsporocyte. Many deeply staining chromatic bodies appear near the periphery of the nucleus.

Figure 3.—Gradual thickening of the threads, some are still slender.

Figure 4.—A later stage in the process of thickening giving a coarse system of uneven threads.

Figure 5.—Early synizesis showing the contraction of the thread system.

Figure 6.—Mid-synizesis. Most of the threads are in closely twisted coils at the side of the large and rather flattened nucleolus.

Figure 7.—Late synizesis. The threads more loose, uniform and thicker.

Figure 8.—Open spireme. The threads show bead-like structures, probably chromomeres.

Figure 9.—A later stage of the open spireme with threads more uniform in thickness.

Figure 10.—Second contraction. Note the central thickening of the threads and the peripheral loops.

Figure 11.—Late second contraction. Chromosomes distinct but much crowded.

Figure 12.—End of the second contraction. The 7 chromosomes are still clustered around the large nucleolus.

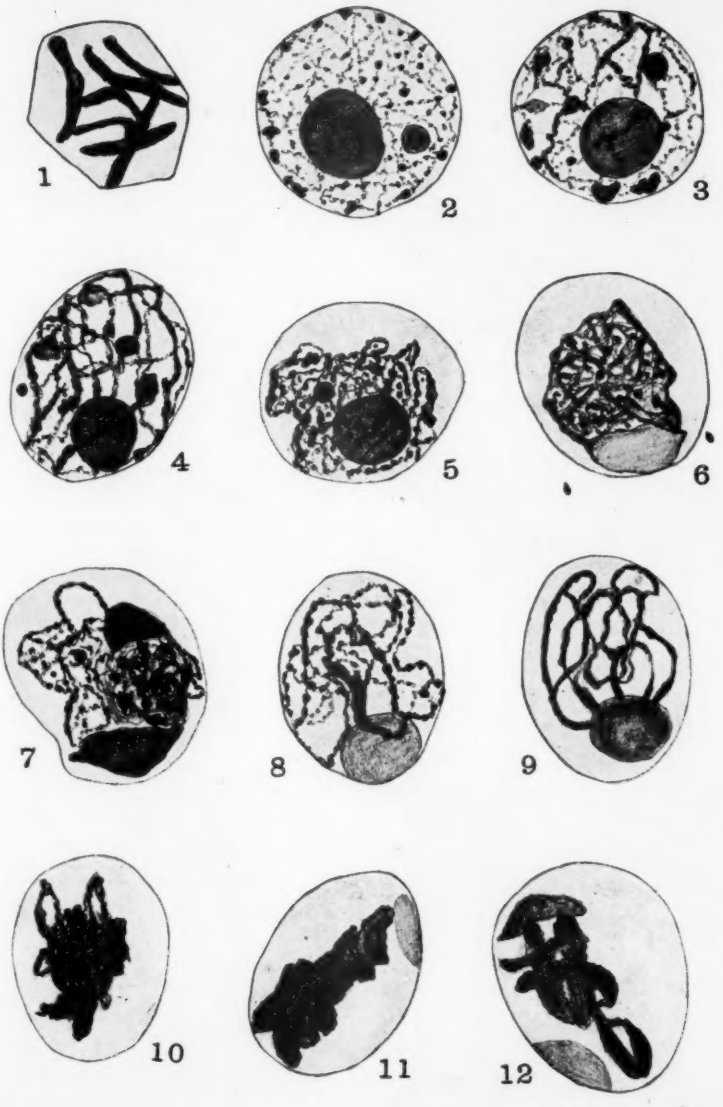
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PLATE 2

FIGURE 13.—Chromosomes shortly after their emergence from second contraction.

FIGURES 14, 15, 16, 17, 18.—Late prophase corresponding to diakinesis. The 7 chromosomes distinct.

FIGURE 19.—Multipolar spindle. The 7 chromosomes gathered in the center of the spindle.

FIGURE 20.—Unipolar spindle. The 7 chromosomes in a plate arrangement.

FIGURE 21.—Unipolar spindle. The 7 chromosomes moving somewhat towards the pole.

FIGURE 22.—Reconstituted nucleus following non-reduction, 7 chromosomes.

FIGURE 23.—Reconstituted nucleus in stage corresponding to interkinesis, 7 split chromosomes.

FIGURE 24.—Metaphase of the homoeotypic mitosis, 7 split chromosomes at the equatorial plate.

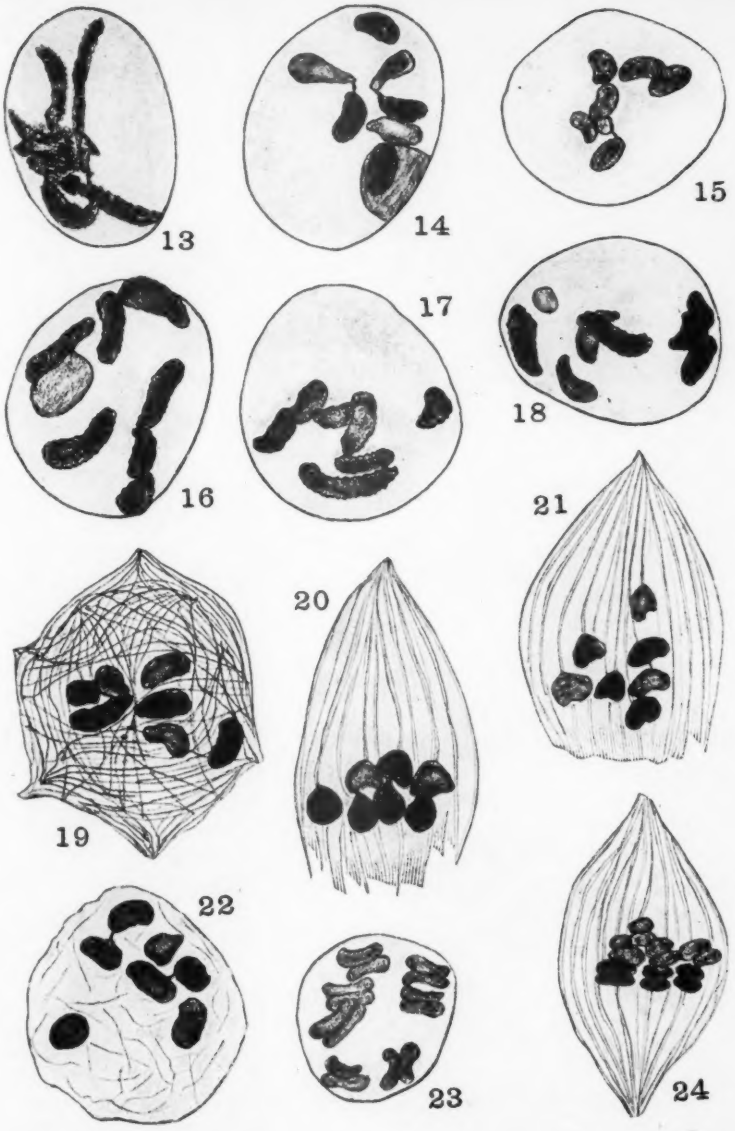
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PLATE 3

FIGURE 25.—Homoeotypic anaphase showing two groups of chromosomes, 7 in each group.

FIGURE 26.—Late homoeotypic anaphase, a group of 7 chromosomes viewed from the pole of the spindle.

FIGURE 27.—Telophase of homoeotypic mitosis.

FIGURE 28.—Diad of young pollen cells within the microsporocyte.

FIGURE 29.—Diad of young pollen cells. A mass of cytoplasm has been pinched off and lies at one side.

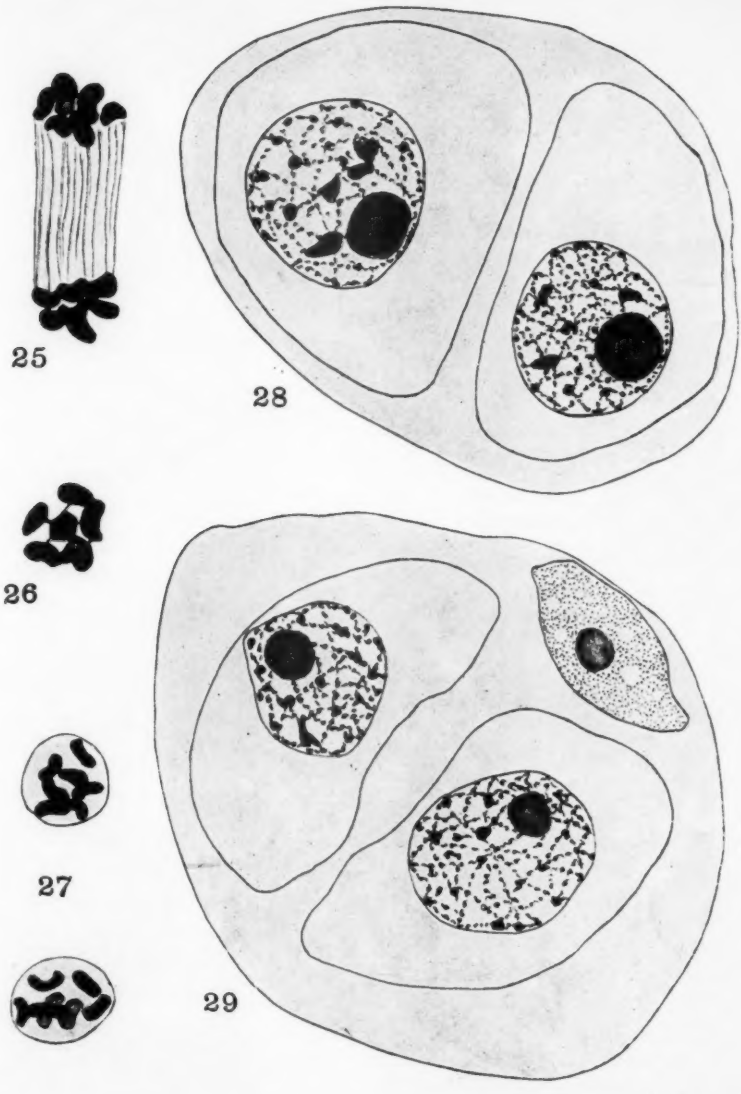
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PLATE 4

Figures 30–35 show irregular distribution of chromosomes during meiosis which will result in shrivelled pollen grains.

FIGURE 30.—Heterotypic anaphase; 6 chromosomes passing to one pole and 1 to the other.

FIGURE 31.—Late heterotypic anaphase; 5 chromosomes at one pole and 2 at the other.

FIGURE 32.—Heterotypic anaphase; 5 chromosomes passing to one pole and 2 to the other.

FIGURE 33.—Heterotypic anaphase; 4 chromosomes passing to one pole and 3 to the other.

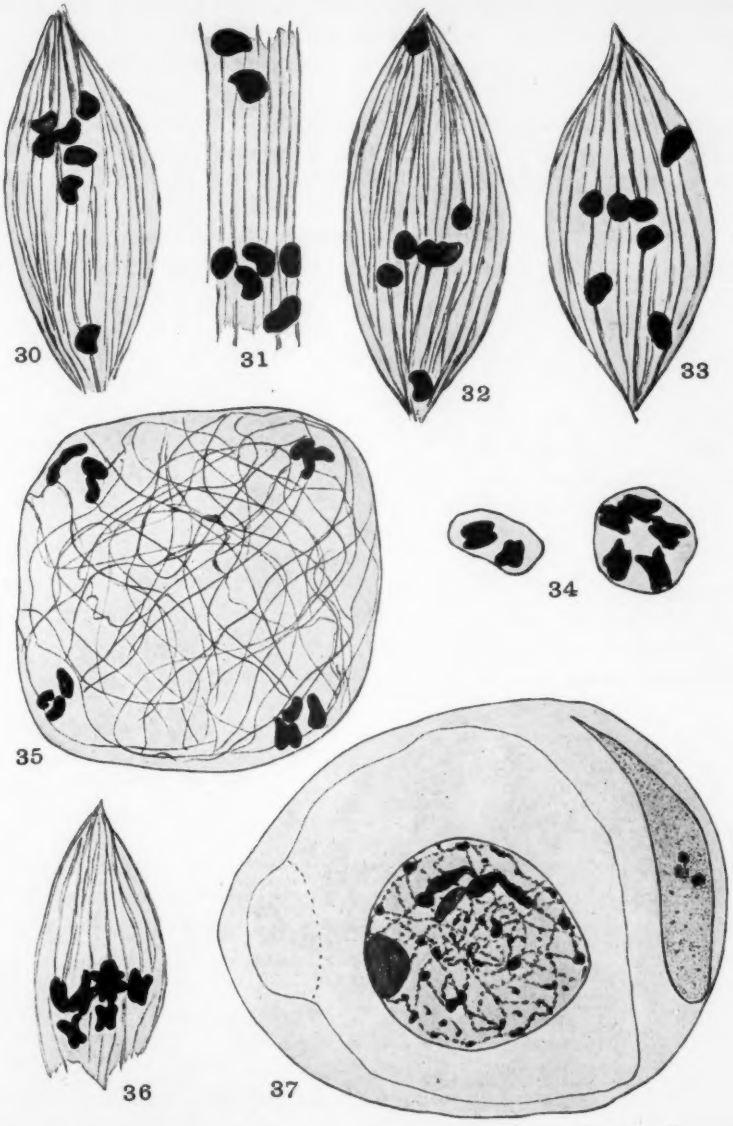
FIGURE 34.—Interkinesis with split chromosomes; five bivalents in one nucleus and two in the other.

FIGURE 35.—Homoeotypic anaphase showing irregular distribution of the 14 chromosomes, two nuclei with 3 and two nuclei with 4 chromosomes.

Figures 36 and 37 show the possible formation of giant pollen grains with 14 chromosomes, a double haploid set.

FIGURE 36.—A unipolar spindle in the homoeotypic mitosis following non-reduction in the heterotypic; 7 split chromosomes, remaining together, will produce a nucleus with 14 chromosomes.

FIGURE 37.—A giant pollen cell accompanied by a mass of pinched off cytoplasm. Such a cell will possibly produce a giant pollen grain with 14 chromosomes.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typic metaphase of *franciscana*, but there is only one spindle in the sporocyte. Anaphase follows, plate 3, figure 25, and the sets of daughter chromosomes move to the poles, figure 26, to organize two daughter nuclei, figure 27, each with 7 chromosomes.

The two nuclei enlarge, pass into a resting condition, the cytoplasm divides, figure 28, and two pollen grains are developed within the microsporocyte. Figure 29 shows the same result with the complication that a small mass of cytoplasm has separated from the two daughter cells. MATSUDA (1928) reports for Petunia similar divisions leading to small masses of cytoplasm without nuclei. These pollen grains are haploid and

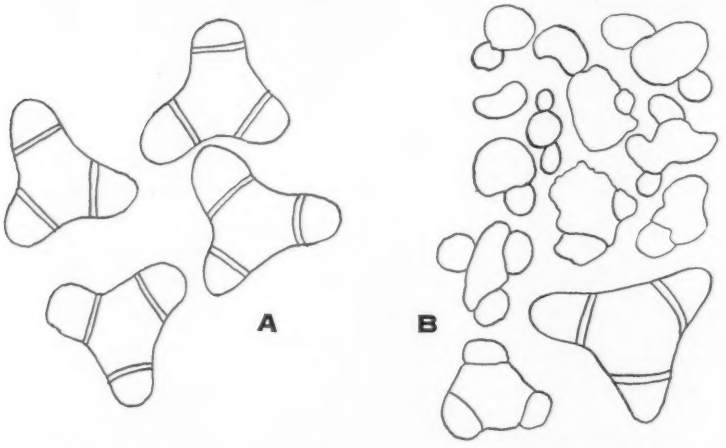

FIGURE 3.—A. Pollen of *Oenothera franciscana*. B. Pollen of the haploid, pointed tips; one good grain is shown among shriveled examples.

they are believed to constitute the fertile pollen which produces *franciscana* zygotes when pointed tips is selfed and when *franciscana* is pollinated by pointed tips. These good pollen grains are of the same size as those of *franciscana*, text figure 3B.

Most of the pollen grains, when produced at all by pointed tips, are shriveled, text figure 3B. Such samplings and estimates as have been made indicate that rarely more than 10 percent of the pollen appears normal in the small amount that an anther may produce. The shriveled grains are to be explained through irregular distribution of chromosomes during meiosis giving grains with less than 7 chromosomes as illustrated

in the series of plate 4 figures, 30–35, which will now be described. In pointed tips there is of course very much more of this material than that which results in pollen grains with the haploid set of 7 chromosomes.

The irregularities of distribution come during the heterotypic mitosis when bipolar spindles are formed and the 7 chromosomes are separated in the possible assortments of 1 and 6,2 and 5,3 and 4. All of these possible results of segregation have been observed and they are illustrated in figures 30–33; the attachments of spindle fibers show the direction of chromosome movement. Figure 30 presents the distribution of 1 and 6, figures 31 and 32 the distribution of 2 and 5, and figure 33 the distribution of 3 and 4.

The results of these divisions are microsporocytes containing a larger and a smaller nucleus. Figure 34 illustrates a case following a segregation of chromosomes, 2 and 5. During the interkinesis the chromosomes split lengthwise, figure 34, and the daughter chromosomes are distributed in the usual way by the homoeotypic mitosis, figure 35, which generally ends with the formation of 4 cells that become abortive pollen grains.

Of particular interest in this material of pointed tips was the discovery of behavior which might lead to the formation of giant pollen grains with 14 chromosomes, a double haploid set. In figure 36 is shown a unipolar homoeotypic spindle with its 7 split chromosomes. This condition must have come from a one-nucleate sporocyte following a suppressed heterotypic mitosis, after which the chromosomes had split as illustrated in figure 23. The condition then followed a unipolar heterotypic spindle such as is shown in figures 20 and 21. Such a failure of the homoeotypic mitosis to distribute the halves of the 7 split chromosomes would result in a giant cell with 14 chromosomes which might become a pollen grain. A single example of such a cell was observed, figure 37, and in this case a small mass of cytoplasm had been pinched off at one side presenting behavior similar to that shown in figure 29. Through such a history might arise in haploid plants from time to time giant pollen grains produced singly in the microsporocytes and carrying two haploid sets of chromosomes. A triploid plant would result through the fertilization of a haploid egg from such a pollen grain and a tetraploid plant through the fertilization of an egg of like constitution.

DISCUSSION

The cytology of the Datura haploids has been described by BELLING and BLAKESLEE (1923, 1927). The 12 chromosomes show no attraction for one another at metaphase of the heterotypic mitosis in the micro-

sporocytes but either move at random to the poles (in assortments of 1 and 11, 2 and 10,3 and 9,4 and 8,5 and 7,6 and 6), or there is non-reduction. When there is segregation the groups of chromosomes after a short interphase pass through a homoeotypic mitosis where each divides and the halves are distributed in the usual manner. Such microsporocytes form 4 small microspores, usually 2 equal and smaller and 2 equal and larger. Polyspores are developed when irregularities during anaphase of the first division give chromosomes independent of the main groups and these organize very small cells in addition to the 4 principal cells. All of these small grains constitute the mass of the abortive pollen which is developed. Non-reduction takes place when the 12 chromosomes split and the halves are distributed in two sets, 12+12, and two pollen grains result each with the haploid set of chromosomes. These constitute the good pollen grains which make up about 12 percent of the total product. The good pollen grains are about the same size as the grains from the diploid Datura. Haploids selfed give diploids as would be expected.

The haploid Oenothera appears to follow the same history as the haploid Datura. Its 7 chromosomes show the same irregular distribution by the heterotypic mitosis in assortments of 1 and 6,2 and 5,3 and 4. The good pollen grains are likewise found 2 in a microsporocyte as the result of one mitosis which distributes the halves of the 7 chromosomes which split. This mitosis in Oenothera is the homoeotypic division, the heterotypic mitosis being suppressed because a bipolar spindle is not developed. BELLING and BLAKESLEE do not take a clear position on the homology of the single mitosis in Datura whether heterotypic or homoeotypic. We suspect it to be the homoeotypic mitosis as in Oenothera and think that they have failed to recognize the suppression of the heterotypic. This might readily happen in studies on iron-acetocarmine preparations which do not show spindle structure.

CHIPMAN and GOODSPEED (1927) give an account of meiosis in the microsporocytes of the haploid from *Nicotiana tabacum* var. *purpurea*, one of the two haploids reported by CLAUSEN and MANN (1924). This haploid (24 chromosomes) was female sterile but some viable pollen is produced. There is a stage of synizesis followed by pachynema which segments into the haploid set of chromosomes which do not pair at diakinesis. Bipolar spindles are often formed resulting in a random distribution of the 24 chromosomes some of which occasionally pass into the cytoplasm. Univalents sometimes divide during the heterotypic mitosis and when rarely all 24 divide there results a giant spindle and the formation of diads which might develop into pollen grains with the full set of

haploid chromosomes. There was some evidence of the occasional suppression of the heterotypic spindle and the gathering of the haploid set of chromosomes into a single nucleus as occurs after the unipolar spindle of our haploid Oenothera. A normal homoeotypic mitosis following such behavior would give diads with the full set of haploid chromosomes. A peculiar feature of the Nicotiana haploid, as also of the haploid wheat, is the precocious division of the univalents at heterotypic metaphase which we have not found in Oenothera. In other respects there seems to be a fairly close agreement in meiotic behavior between the haploid Nicotiana and the haploid Oenothera.

GAINES and AASE (1926) in their account of the haploid wheat were the first to publish a detailed description of the cytology of a haploid spermatophyte. The haploid (21 chromosomes) came from seed of a hybrid wheat No. 128, *Triticum compactum humboldtii* Kcke. (42 chromosomes) after an attempt to pollinate by *Aegilops cylindrica*. The haploid was not to be distinguished from the female parent No. 128 until the time of flowering when peculiarities characteristic of sterility appeared. The plant was about 99.8 percent seed sterile. There was no pairing of chromosomes during meiosis in the microsporocytes because there were probably three dissimilar sets of 7 chromosomes each. The chromosomes are generally distributed irregularly during the heterotypic mitosis but sometimes the chromosomes divide and the halves pass in an orderly manner to opposite poles, simulating a homoeotypic mitosis, or there may be a mixture of the two processes. The homoeotypic mitosis continues the disorderly distribution of the chromosomes giving many irregularities and forms of polyspory. No normal pollen grains were observed to develop although such might rarely be formed. An uncommon peculiarity was observed in the fusion of adjacent microsporocytes after which giant spindles may be developed including the chromosomes of two or more nuclei; these do not pair and are distributed at random. This would seem to be behavior similar to the process of "endo-duplication" described by JÖRGENSEN (1928). From such fusions come giant pollen grains which may occasionally be found in the mixture of abnormalities produced. Except for the extraordinary fusions of the microsporocytes and the early division of the chromosomes before their distribution by the heterotypic mitosis most of the irregularities of this haploid wheat are present in our haploid Oenothera which in addition gave us the history of the small amount of fertile pollen produced.

Very different in some important respects from the other cytological accounts of haploids is the interesting description of some Solanum

haploids by JÖRGENSEN (1928). The pollen tube of *Solanum luteum* may enter the embryo sac of *S. nigrum* and discharge its two sperm nuclei but these fail to fuse with the egg and endosperm nucleus and finally disintegrate. From some of the unfertilized eggs embryos begin to develop and there can scarcely be doubt that the seeds which produce haploids come from such parthenogenetic development. During meiosis in the microsporocyte the 36 chromosomes of the haploid at diakinesis show some degree of pairing, the number of pairs ranging from 3 to 11 or 12. The larger number of pairs suggests a reduction division of the 12_2+12_1 type. At the heterotypic metaphase only the bivalents are constantly present on the equatorial plate, the univalents being scattered through the cell and most often in the polar regions. The number of bivalents on the plate ranges from 3 to 12 with the numbers 5 to 8 most frequent. Univalent chromosomes lying near the plate may divide and their products can be recognized at the homoeotypic metaphase by their small size. The chromosomes pass irregularly to the poles in numbers ranging from 15 to 22 with 18 most frequent. The homoeotypic mitosis more commonly presents 18 chromosomes at metaphase; these divide and anaphase proceeds regularly giving 4 pollen grains each as a rule with 18 chromosomes. Meiosis in the megasporocyte is peculiar in that more of the chromosomes during the heterotypic mitosis pass to the micropylar end of the cell. Since the embryo sac develops from the chalazal megaspore it receives a nucleus with the smaller number of chromosomes (14 to 16) which apparently are not enough for further development since the nucellus soon breaks down. A striking peculiarity of the Solanum haploids is the pairing of certain chromosomes in the heterotypic mitosis. This, as JÖRGENSEN points out, suggests that the 36 chromosomes of the haploid constitute a group composed of 3 sets of 12 each of which 2 sets contain homologues of sufficient similarity to bring them together in a true synapsis. Such behavior would not be expected and does not occur in Datura, Nicotiana or Oenothera where the haploid count is the basic chromosome number of the genus.

The most interesting feature of the process leading to the development of the fertile pollen of the haploid Oenothera is the clear cut suppression of the heterotypic mitosis through failure to develop a normal spindle with the result that a reconstituted nucleus takes the seven chromosomes which then divide and the halves are distributed by a homoeotypic mitosis to form two pollen grains in each microsporocyte. There is then no reduction division and the fertile pollen carries the somatic set of seven chromosomes characteristic of the haploid. ROSENBERG (1917) first de-

scribed such a history for *Hieracium laevigatum* and *H. lacerum* and called it a "semi-heterotypic" division including under the term also the cases where chromosomes without conjugation are distributed irregularly by the heterotypic mitosis to give diads with various chromosome counts. This latter behavior is also found in the haploid Oenothera and gives the large mass of shriveled pollen grains present.

The term "semi-heterotypic" does not seem to us fortunate since the heterotypic mitosis either fails entirely to operate or performs irregularly; in neither case is it a half expressed division. We prefer to call the behavior of the first type suppression of the heterotypic mitosis and it is of course a very important modification of sporogenesis since it results in fertile pollen with the set of chromosomes characteristic of the parent. ROSENBERG was quick to point out that suppression of the heterotypic mitosis permits the doubling of chromosome numbers through the union of gametes carrying the somatic sets of the parents. Thus, as from fertile haploids come diploids so from diploids might come tetraploids. His earlier work was on parthenogenetic Hieracia but in later papers (ROSENBERG 1926, 1927) he develops this idea and suggests the possible origin of tetraploid hybrids through the union of gametes produced with nonreduction.

Material support for ROSENBERG'S views on the origin of tetraploid hybrids has appeared in the interesting cross of Raphanus × Brassica recently described by KARPECHENKO (1927). The F_1 hybrids may form diploid pollen grains by the suppression of the heterotypic mitosis. Tetraploid pollen grains may arise through two steps:—(1) A tetraploid group is established by the association of two nuclei in a microsporocyte and the gathering of the two diploid sets of chromosomes through the fusion of the two heterotypic spindles; (2) The failure of the two combined heterotypic spindles to bring about segregation leads to a reconstituted nucleus with the tetraploid group, and tetraploid diads are formed following a homoeotypic mitosis. Unions of haploid, diploid and tetraploid gametes in various combinations, complicated by some numerical irregularities of chromosome distribution, give possibilities of a large assortment of polyploids which were in part realized. Among these the tetraploids from the union of diploid gametes were noteworthy as fertile plants showing no segregation and with characters of a species distinct from both parents. They show one way through which stable and fertile tetraploid hybrids may arise in sharp contrast to the "indirect chromosome binding" of WINGE (1917,1925), and the somatic tetraploidy by "endo-duplication" of JÖRGENSEN (1928). It is interesting to note how

complicated has become the subject of the origin of polyploids, with a number of possible methods probably present in different groups of plants.

Haploids carrying only non-homologous chromosomes have a peculiar interest in genetics because there can be no question of heterozygosity to complicate, if they are fertile, a study of their progeny. When selfed their seed should give homozygous diploids unless gene mutations take place or irregularities of chromosome distribution produce chromosomal sports which may be checked by cytological studies. Both chromosomal sports and gene mutations in diploids from haploids have been noted in Datura (BLAKESLEE and others 1927). Two gene mutations, curled and tri-carpel, have been established. They are rare and recessive and the evidence indicates that the mutations probably occur in the egg or pollen grain of the haploid parents. In the selfed lines from the first plant of pointed tips, table 1, an interesting plant, *red elongate*, with important distinguishing characters appeared in three cultures (27.41, 27.42 and 27.43). One family (28.35) has been grown from red elongate selfed; it consisted of 185 *franciscana*, 20 red elongate, 1 pointed tip and 1 broad-leaved dwarf. The result suggests irregularities of chromosome distribution and offers an interesting subject for study. Progeny from another selfed plant of pointed tips out of the cross *franciscana*×*franciscana sulfurea* dwarf, table 3 (culture 27.45), gave two remarkable plants of a wholly new type named *light green* which has pollen almost wholly perfect. From light green selfed one family (28.37) has been grown consisting of 113 plants all true to the parent type. This behavior suggests a gene mutation and it should be studied in contrast to red elongate.

SUMMARY

1. A haploid sport, named pointed tips, is thrown by the homozygous *Oenothera franciscana*. The plants are of about half the stature of *franciscana* with all organs proportionally smaller. Pollen is produced in small amounts and frequently flowers develop none at all, the anthers becoming shriveled. The pollen contains from 60 to 90 percent of shrunken and empty grains. Some fertile ovules are developed and the plant when selfed sets a small amount of seed.

2. The haploid thus far has appeared only four times in a series of generations of *O. franciscana* in a ratio of about 1:1000. It has also been thrown from crosses of *franciscana* to certain derivatives.

3. Pointed tips selfed, tables 1 and 2, gives a progeny almost wholly of the parent type *franciscana*, but a few plants of pointed tips appear and occasionally other forms. Of the latter, red elongate and light green merit attention and further study.

4. Pointed tips backcrossed to *franciscana*, table 3, gave only *franciscana* but in large cultures an occasional haploid would be expected.

5. Pointed tips is also recorded from a large culture of *Oenothera Hookeri*, a homozygous form very closely related to *O. franciscana*.

6. In all cases the haploid may be interpreted as arising from the parthenogenetic development of an egg. This interpretation finds support in the fact that pointed tips selfed and backcrossed to *franciscana* gives almost wholly *franciscana* showing that its gametes must be of the *franciscana* genotype.

7. The few fertile pollen grains of the haploid are developed as the result of the suppression of the heterotypic mitosis. The prophases are as in *franciscana* except that there is no pairing of the 7 chromosomes at diakinesis. The heterotypic spindle fails to attain the bipolar condition although one pole is generally evident. The chromosomes are not distributed but remain in a group and a nucleus is reconstituted which corresponds to that in the period of interkinesis of normal meiosis. In this nucleus the chromosomes split exactly as in interkinesis. Then comes a homoeotypic mitosis which distributes the halves of the split chromosomes to give two nuclei with 7 chromosomes each, and two fertile pollen grains are developed in each microsporocyte.

8. The large mass of shriveled and sterile pollen grains results from the irregular distribution of the 7 chromosomes by the heterotypic mitosis in assortments of 1 and 6,2 and 5,3 and 4. In this manner the microsporocyte comes to contain a larger and a smaller nucleus neither of which has the full complement of 7 chromosomes. During interkinesis the chromosomes split and the halves are distributed as usual by a homoeotypic mitosis with the result that four nuclei are formed all lacking the full set of chromosomes. Cell division usually gives four small pollen grains which become shrunken and empty of contents.

9. Very rarely a homoeotypic spindle following the suppression of the heterotypic mitosis will itself fail to reach full development. In such cases the 7 split chromosomes of the period corresponding to interkinesis enter a reconstituted nucleus and a single giant pollen grain with 14 chromosomes may be produced by the microsporocyte.

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FORMULAE AND TABLES FOR CALCULATING LINKAGE INTENSITIES[1]*

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INTRODUCTION

At present many different methods are used by the various workers in the field of Genetics to calculate linkage intensities from F_2 data.

In some cases two or more methods applied to the same data give widely different results.

A general method which is satisfactory for all ordinary linkage problems would help greatly to reduce the confusion which exists now because of the great number of methods being used.

BATESON and PUNNETT (1911) were the first to give a method for measuring linkage intensity. Inspection was relied on to determine the closeness of fit between calculated and observed ratios. COLLINS (1912, 1924) made the coefficient of association (YULE, 1900) the basis of a method for calculating linkage intensities. BRIDGES (1914) used the same method as COLLINS (1912). EMERSON (1916) presented a formula which FISHER (1928) has referred to as the additive method and CASTLE (1916) used the same basis for his method. WOODWORTH (1923) and BRUNSON (1924) developed formulae for cases in which duplicate or complementary factors were involved which were based on EMERSON'S method. HOR (1924), BABCOCK and CLAUSEN (1927), ALBERTS (1926) and KAPPERT (1927) have presented still other formulae.

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WELLENSIEK (1927) has given two methods for calculating the actual gametic F_2 series from a given zygotic series. The first method gives the actual ratio separately for each of the four types of gametes but does not show differences between the male and female gametic series. The second method makes it possible to calculate the actual gametic ratio of the four types of gametes for both male and female gametic series.

OWEN (1928) used the product moment correlation coefficient in developing formulae for calculating linkage intensities. His formulae have the added advantage of convenient algebraic manipulation. Further information regarding a linkage problem may be obtained with very little additional calculation.

HALDANE (1919) presented both a formula for calculating the crossover percentage from F_2 data and a formula for obtaining the probable error. Recently FISHER (1928) and FISHER and BALMUKAND (1928) have given a critical analysis of several of the above mentioned formulae. Methods are given by which the probable error formulae may be determined (FISHER 1928) and a number of ways for comparing the relative efficiency of different formulae. A further comparison of the relative efficiency of certain of the current methods of calculating linkage intensities is given in this paper. Tables are appended which greatly simplify the calculation by the product method of linkage intensities and their probable errors.

EFFICIENCY OF SEVERAL DIFFERENT FORMULAE

In order to be most useful a formula for calculating linkage intensities should be easy to use, it should be disturbed as little as possible by differential mortality of gametes or zygotes, and it should be statistically efficient. The term efficient is here used in the statistical sense as meaning that the formula has a probable error as small as possible. The comparative efficiency of other formulae may be determined by dividing the variance (squared standard deviation) of an efficient formula by the variance of the formula in question. The efficiency of several formulae will be discussed

In the discussion in this paper the symbol p will be used to designate the crossover percentage, expressed as a decimal fraction, where the cross was made in the repulsion phase. In repulsion crosses p will then vary from 0 to about .50. In the coupling phase p will represent the percentage of parental combinations, expressed as a decimal fraction. In that case p will vary from about .50 to 1.00 and $1-p$ will then be the crossover percentage. All formulae presented in this paper will use the symbol p in the manner defined above.

FISHER (1928) and FISHER and BALMUKAND (1928) state that the maximum likelihood method (for which they propose the symbol T_4, where $T_4=p^2$) will in all cases have a probable error, in the theory of large samples, as small as possible. This method consists in multiplying the logarithm of the number expected in each of the four F_2 phenotypes by the number observed, summing for the four classes and finding the value of p^2 which will make this sum a maximum. It is stated further that HALDANE (1919) could have arrived at his formula only by using the maximum likelihood solution. This method can then be used as a standard of comparison, from the probable error standpoint, for other methods.

FISHER (1928) and FISHER and BALMUKAND (1928) have shown that EMERSON'S method, called the additive method by these writers, is efficient *only* for close linkage in the coupling phase. The fraction of information utilized for various crossover percentages by the additive or EMERSON method may be obtained by dividing the variance of the maximum likelihood method by the variance of the additive method, which leads to the formula $2p^2(2+p^2)/(1+2p^2)(1+p^2)$, where p is used as previously defined. Substituting the values $p=.90$ (10 percent crossing over in the coupling phase) and $p=.10$ (10 percent crossing over in repulsion) in the above formula, we find that EMERSON'S formula is 96 percent efficient at 10 percent crossing over in the coupling phase and 4 percent efficient at 10 percent crossing over in the repulsion phase; that is, with 10 percent crossing over in repulsion it utilizes but 4 percent of the information which would be utilized by the maximum likelihood method or the product method. At 50 percent crossing over the additive or EMERSON method is 60 percent efficient. This emphasizes the errors which might be encountered by using EMERSON'S method as a general method, particularly in the repulsion phase.

FISHER (1928) and FISHER and BALMUKAND (1928) give a product method (for which the symbol T_3 is proposed, where $T_3=p^2$) which is equivalent to the coefficient of association method developed by COLLINS (1912, 1924) and BRIDGES (1914). It has the same probable error as the maximum likelihood or HALDANE'S method. Therefore it is of equal efficiency with the maximum likelihood method, which we have previously accepted as our standard for judging the efficiency of other formulae.

It has been shown by COLLINS (1924) and OWEN (1928) that the coefficient of association method (which is equivalent to the product method), of all the methods compared, seems to be affected the least by differential mortality of gametes or zygotes, although OWEN'S (1928) correlation coefficient method is but slightly inferior. The ease of calculating linkage in-

tensities by the product method from the calculated tables presented in this paper will be discussed later. It seems, therefore, that the product method is the best general method available since it is the easiest to use when suitable tables are available, it is affected the least by differential mortality, and it has probable error, in the theory of large samples, as small as possible.

THE PRODUCT METHOD

Given the four observed F_2 phenotypic classes as AB, Ab, aB and ab and designating these by a, b, c, and d respectively, FISHER's product method formula for two 3:1 ratios is $ad/bc=p^2(2+p^2)/(1-p^2)^2$. In calculating linkage intensities by this method, the observed frequencies for the four classes, a, b, c, and d are substituted in this formula and the value of p determined.

Since the product method seems to be the best general method available, because of its ease of calculation when tables are available, the magnitude of the probable error which is as small as possible, and the fact that it is disturbed the least by differential mortality of gametes or zygotes, it seems desirable to extend the method to the more complex ratios in which duplicate or complementary factors are involved. Such formulae are given in table 1. The formulae which have been available for these ratios were based either on EMERSON's method, which in the case of two 3:1 ratios has been shown to be inefficient except for close linkage in the coupling phase, or on OWEN's correlation coefficient method for which no method for developing probable error formulae has yet been presented. The determination of p for any of the factor relationships dealt with in table 1 then simply resolves itself into a solution of the proper quadratic equation. In these formulae a linkage is assumed between but one factor pair for each of the character pairs concerned.

Formulae for the probable errors of linkage intensities calculated by the product method may also be developed. FISHER (1928) and FISHER and BALMUKAND (1928) give the probable error of p calculated from two 3:1 ratios as $.6745\sqrt{(1-p^2)(2+p^2)/2(1+2p^2)N}$ where N is the total frequency. The formula for the probable error of p calculated from a backcross is usually given as $.6745\sqrt{p(1-p)/N}$. FISHER (1928) has given a general method by which the probable errors of other product method formulae may be determined. Such probable error formulae are given in table 1. The probable error concept applied to linkage problems should furnish the sound basis for judging the reliability of calculated linkage intensities which has been lacking so often in the past.

TABLE 1

Formulae for the calculation of crossover values and their probable errors based on the product method. In repulsion p is the crossover percentage and in coupling $1-p$ is the crossover percentage, expressed as decimal fractions.

PHENOTYPIC RATIOS	FORMULAE TO CALCULATE p	PROBABLE ERROR
3:1 and 1:1	$\frac{ad}{bc}=\frac{p+p^2}{2-3p+p^2}$	$.6745\sqrt{\frac{2p(2-p)(1-p^2)}{N(1+2p-2p^2)}}$
3:1 and 3:1	$\frac{ad}{bc}=\frac{2p^2+p^4}{1-2p^2+p^4}$	$.6745\sqrt{\frac{(1-p^2)(2+p^2)}{2N(1+2p^2)}}$
9:7 and 3:1	$\frac{ad}{bc}=\frac{2+7p^2+3p^4}{6-9p^2+3p^4}$	$.6745\sqrt{\frac{(1+3p^2)(1-p^2)(4-p^4)}{3Np^2(5+2p^2-4p^4)}}$
27:37 and 3:1	$\frac{ad}{bc}=\frac{14+25p^2+9p^4}{30-39p^2+9p^4}$	$.6745\sqrt{\frac{(1-p^2)(2+p^2)(10-3p^2)(7+9p^2)}{27Np^2(9+2p^2-4p^4)}}$
15:1 and 3:1	$\frac{ad}{bc}=\frac{11p^2+p^4}{4-5p^2+p^4}$	$.6745\sqrt{\frac{(1-p^2)(4-p^2)(11+p^2)}{N(11+2p^2-4p^4)}}$
63:1 and 3:1	$\frac{ad}{bc}=\frac{47p^2+p^4}{16-17p^2+p^4}$	$.6745\sqrt{\frac{(1-p^2)(16-p^2)(47+p^2)}{N(47+2p^2-4p^4)}}$
9:7 and 9:7	$\frac{ad}{bc}=\frac{20+28p^2+9p^4}{36-36p^2+9p^4}$	$.6745\sqrt{\frac{(10+9p^2)(4-p^4)}{18Np^2(3+2p^2)}}$
9:7 and 15:1	$\frac{ad}{bc}=\frac{11+34p^2+3p^4}{27-30p^2+3p^4}$	$.6745\sqrt{\frac{(1+3p^2)(9-p^2)(1-p^2)(11+p^2)}{6Np^2(13+p^2-2p^4)}}$
15:1 and 15:1	$\frac{ad}{bc}=\frac{56p^2+p^4}{16-8p^2+p^4}$	$.6745\sqrt{\frac{(4-p^2)(56+p^2)}{2N(7+2p^2)}}$

COMPARATIVE RELIABILITY OF LINKAGES CALCULATED FROM BACKCROSSES AND FROM F_2 DATA

Since the probable error of a linkage intensity calculated from a backcross will, in all cases, be less than from the same size of population in F_2, the backcross method may be used as a standard of comparison for F_2 data. In figure 1 is shown graphically the relative accuracy of backcross data compared with F_2 data. The comparison is made on the basis of the number of times as many individuals needed to establish a linkage intensity with the probable error, from F_2 data as from a backcross, the product method being used to determine the linkage from the F_2 data. It is seen that for the coupling phase F_2 data are but slightly in-

ferior to backcross data, especially for close linkage. At 50 per cent, crossing over it would require 2.25 times as many individuals in F_2 to obtain the same reliability as from a backcross. This fact was first pointed out by HALDANE in 1919. F_2 repulsion data are much less reliable than F_2 coupling, especially for close linkages. As an illustration, a determination

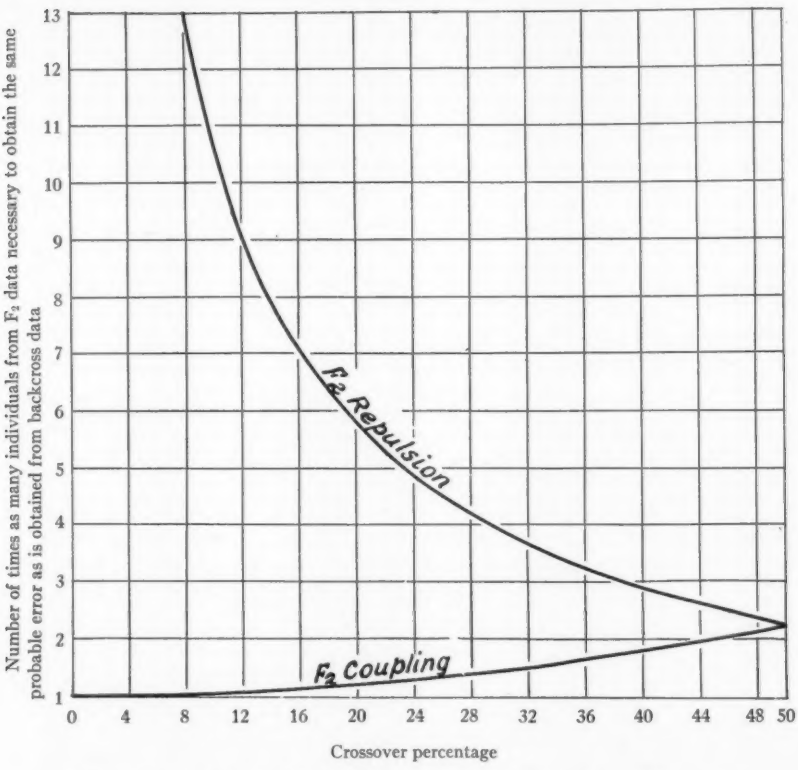

FIGURE 1.—Graph showing the relative reliability of F_2 and backcross data for determining linkage intensities.

of 10 percent crossing over based on 1,000 individuals from a backcross would be as reliable as if the same were obtained from 1,130 individuals from F_2 data from a cross in the coupling phase or 10,830 individuals from F_2 repulsion. This emphasizes again the relative reliability of F_2 data for the determination of close linkage in the coupling phase as contrasted

with the repulsion phase. The repulsion phase is only as reliable as the coupling phase for 50 percent crossing over. For close linkage the repulsion phase is very inferior to coupling.

It can be concluded then that in linkage studies where backcrossing is difficult or time consuming that linkages may be determined accurately from F_2 data, the accuracy depending on whether the cross was made in the coupling or repulsion phase and whether a close or a loose linkage is found. If the factors entered in the coupling phase in a given cross the number of times as many individuals in F_2 necessary to obtain the same reliability as from a backcross would vary from 1 to 2.25. If the cross were made in the repulsion phase, the number of times as many individuals in F_2 necessary to obtain the same reliability as from a backcross would vary from 2.25 to ∞ as the linkage varied from 50 to 0 percent.

RELIABILITY OF LINKAGE DETERMINATIONS WHEN COMPLEMENTARY OR DUPLICATE FACTORS ARE INVOLVED

Having established the relative reliability of F_2 data as compared with backcross data, it would seem logical to determine next the relative reliability of linkage determinations when three or four factor pairs are concerned as compared with only two factor pairs.

In figure 2 is shown graphically the relative reliability of linkage intensity calculations from 9:7 and 3:1, 27:37 and 3:1, 15:1 and 3:1, or 63:1 and 3:1 ratios, using the probable error of the same crossover percentage for two 3:1 ratios as the standard of comparison. The efficiency of these more complex ratios is given in terms of the number of times as many individuals necessary to establish a linkage with the same probable error as would be obtained from two 3:1 ratios. It will be seen that linkage intensities can be determined fairly accurately when 9:7 and 3:1 or 27:37 and 3:1 ratios are involved if the cross is made in the coupling phase. If the cross is one of repulsion the probable errors are much larger than the probable errors for F_2 repulsion in the case of two 3:1 ratios, which are themselves very inferior to F_2 coupling as shown in figure 1. It is clear, as shown by the graph, that linkage intensities calculated from 9:7 and 3:1 or 27:37 and 3:1 ratios and based on F_2 repulsion data are very unreliable for close linkages unless very large numbers are obtained. This emphasizes the decided superiority of the coupling over the repulsion phase for three or four factor problems when complementary factors are involved.

Linkage intensities calculated from F_2 data when duplicate factors deter-

mine one of the character pairs are also less reliable when the repulsion phase is used than with the coupling phase although the difference between the coupling and repulsion phases is less here than when two 3:1 ratios are involved. Larger populations are needed to obtain the same reliability when duplicate factors are involved as when only two 3:1 ratios are involved.

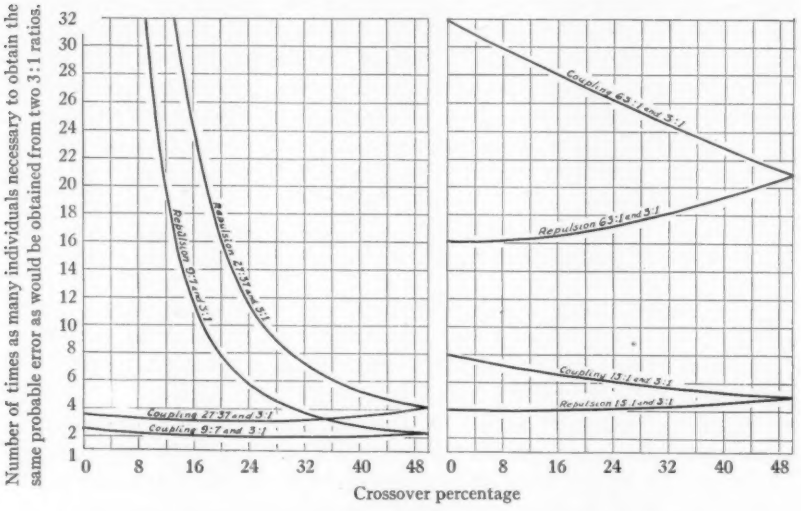

FIGURE 2.—Graph showing relative reliability of linkage intensities calculated from ratios in which more than 2 factors are involved as compared with determinations from two 3:1 ratios.

TABLES FOR CALCULATING LINKAGE INTENSITIES

In calculating linkage intensities from the formulae given in table 1, it is necessary to reduce the proper equation to the form of a quadratic, solve for p^2 and extract the square root. While this is not difficult, it may be rather laborious under some conditions and will always be time consuming. Fortunately the product method lends itself readily to the calculation of tables from which linkage intensities and their probable errors may be determined with the minimum of effort. FISHER and BALMUKAND (1928) gave a small table of this kind. More extended tables have been calculated and are presented here. The method of using these tables will be illustrated.

TABLE 2

Constants to facilitate the calculation of linkage intensities, by the product method, when each of two character pairs is determined by a single factor difference. Constants are given also to be used in obtaining probable errors for 3:1 and 3:1 ratios.

CROSSOVER VALUE	RATIO OF PRODUCTS		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN PROBABLE ERROR		
	ad/bc REPULSION	*bc/ad* COUPLING	F_2 REPULSION	F_2 COUPLING	BACKCROSS
.005	.00005000	.00003361	.6745	.04771	.04757
.010	.00020005	.0001356	.6744	.06751	.06711
.015	.0004503	.0003076	.6743	.08271	.08199
.020	.0008008	.0005516	.6742	.09555	.09443
.025	.001252	.0008692	.6740	.1069	.1053
.030	.001804	.001262	.6737	.1171	.1151
.035	.002458	.001733	.6735	.1266	.1240
.040	.003213	.002283	.6731	.1354	.1322
.045	.004070	.002914	.6728	.1436	.1398
.050	.005031	.003629	.6724	.1515	.1470
.055	.006096	.004429	.6719	.1590	.1538
.060	.007265	.005318	.6715	.1661	.1602
.065	.008540	.006296	.6709	.1730	.1663
.070	.009921	.007366	.6704	.1796	.1721
.075	.01141	.008531	.6698	.1860	.1777
.080	.01301	.009793	.6691	.1922	.1830
.085	.01471	.01116	.6684	.1982	.1881
.090	.01653	.01262	.6677	.2040	.1930
.095	.01846	.01419	.6670	.2097	.1978
.100	.02051	.01586	.6662	.2153	.2024
.105	.02267	.01765	.6653	.2207	.2068
.110	.02495	.01954	.6644	.2260	.2110
.115	.02734	.02156	.6635	.2312	.2152
.120	.02986	.02369	.6625	.2363	.2192
.125	.03250	.02594	.6616	.2413	.2231
.130	.03527	.02832	.6605	.2463	.2268
.135	.03816	.03083	.6594	.2511	.2305
.140	.04118	.03347	.6583	.2558	.2340
.145	.04434	.03624	.6572	.2605	.2375
.150	.04763	.03915	.6560	.2651	.2408
.155	.05105	.04220	.6548	.2697	.2441
.160	.05462	.04540	.6535	.2741	.2473
.165	.05832	.04875	.6522	.2785	.2503
.170	.06218	.05225	.6509	.2829	.2534
.175	.06618	.05591	.6495	.2872	.2563
.180	.07033	.05973	.6482	.2914	.2591
.185	.07464	.06371	.6467	.2956	.2619
.190	.07911	.06787	.6453	.2998	.2646
.195	.08374	.07220	.6438	.3039	.2672
.200	.08854	.07671	.6422	.3079	.2698
.205	.09351	.08140	.6407	.3119	.2723
.210	.09865	.08628	.6391	.3159	.2747

TABLE 2 (*continued*)

CROSSOVER VALUE	RATIO OF PRODUCTS		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN PROBABLE ERROR		
	ad/bc REPULSION	*bc/ad* COUPLING	F_2 REPULSION	F_2 COUPLING	BACKCROSS
.215	.1040	.09136	.6375	.3198	.2771
.220	.1095	.09663	.6358	.3237	.2794
.225	.1152	.1021	.6341	.3276	.2817
.230	.1211	.1078	.6324	.3314	.2839
.235	.1272	.1137	.6307	.3352	.2860
.240	.1334	.1198	.6289	.3390	.2881
.245	.1400	.1262	.6271	.3427	.2901
.250	.1467	.1328	.6253	.3464	.2921
.255	.1536	.1396	.6234	.3501	.2940
.260	.1608	.1467	.6215	.3537	.2959
.265	.1682	.1540	.6196	.3573	.2977
.270	.1758	.1616	.6177	.3609	.2995
.275	.1837	.1695	.6157	.3645	.3012
.280	.1919	.1777	.6137	.3680	.3029
.285	.2003	.1861	.6117	.3716	.3045
.290	.2089	.1948	.6097	.3750	.3061
.295	.2179	.2038	.6076	.3785	.3076
.300	.2271	.2132	.6055	.3820	.3091
.305	.2367	.2228	.6034	.3854	.3105
.310	.2465	.2328	.6012	.3888	.3119
.315	.2567	.2432	.5991	.3922	.3133
.320	.2672	.2538	.5969	.3955	.3146
.325	.2780	.2649	.5947	.3989	.3159
.330	.2892	.2763	.5925	.4022	.3172
.335	.3008	.2881	.5902	.4055	.3184
.340	.3127	.3003	.5879	.4088	.3195
.345	.3250	.3128	.5857	.4121	.3206
.350	.3377	.3259	.5833	.4153	.3217
.355	.3508	.3393	.5810	.4185	.3228
.360	.3643	.3532	.5787	.4218	.3238
.365	.3783	.3675	.5763	.4250	.3247
.370	.3927	.3823	.5739	.4281	.3256
.375	.4076	.3977	.5715	.4313	.3265
.380	.4230	.4135	.5691	.4345	.3274
.385	.4389	.4298	.5666	.4376	.3282
.390	.4553	.4467	.5641	.4407	.3290
.395	.4723	.4641	.5617	.4438	.3297
.400	.4898	.4821	.5592	.4469	.3304
.405	.5079	.5007	.5566	.4500	.3311
.410	.5266	.5199	.5541	.4531	.3317
.415	.5460	.5398	.5516	.4561	.3323
.420	.5660	.5603	.5490	.4592	.3329
.425	.5867	.5815	.5464	.4622	.3334
.430	.6081	.6034	.5438	.4652	.3339
435	.6302	.6260	.5412	.4682	.3344

TABLE 2 (*continued*)

CROSSOVER VALUE	RATIO OF PRODUCTS		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN PROBABLE ERROR		
	ad/bc REPULSION	*bc/ad* COUPLING	F_2 REPULSION	F_2 COUPLING	BACKCROSS
.440	.6531	.6494	.5386	.4712	.3348
.445	.6768	.6735	.5359	.4741	.3352
.450	.7013	.6985	.5333	.4771	.3356
.455	.7266	.7243	.5306	.4800	.3359
.460	.7529	.7510	.5279	.4829	.3362
.465	.7801	.7786	.5252	.4859	.3364
.470	.8082	.8071	.5225	.4888	.3366
.475	.8374	.8366	.5197	.4916	.3368
.480	.8676	.8671	.5170	.4945	.3370
.485	.8990	.8986	.5142	.4974	.3371
.490	.9314	.9313	.5115	.5002	.3372
.495	.9651	.9651	.5087	.5031	.3372
.500	1.0000	1.0000	.5059	.5059	.3372
.505	1.0362	1.0362	.5031	.5087	.3372
.510	1.0738	1.0736	.5002	.5115	.3372
.515	1.1128	1.1124	.4974	.5142	.3371
.520	1.1533	1.1526	.4945	.5170	.3370
.525	1.1958	1.1942	.4916	.5197	.3368
.530	1.2390	1.2373	.4888	.5225	.3366
.535	1.2844	1.2819	.4859	.5252	.3364
.540	1.3316	1.3282	.4829	.5279	.3362
.545	1.3806	1.3762	.4800	.5306	.3359
.550	1.4317	1.4260	.4771	.5333	.3356
.555	1.4847	1.4776	.4741	.5359	.3352
.560	1.5400	1.5312	.4712	.5386	.3348
.565	1.5975	1.5868	.4682	.5412	.3344
.570	1.6574	1.6446	.4652	.5438	.3339
.575	1.7198	1.7045	.4622	.5464	.3334
.580	1.7848	1.7668	.4592	.5490	.3329
.585	1.8526	1.8316	.4561	.5516	.3323
.590	1.9234	1.8989	.4531	.5541	.3317
.595	1.9972	1.9689	.4500	.5566	.3311
.600	2.0742	2.0417	.4469	.5592	.3304

By calculating tables giving the values of the ratio of products ad/bc or bc/ad for different values of p, the labor of determining linkage intensities can be materially reduced. Such aids for the rapid determination of linkages from 3:1 and 3:1, 9:7 and 3:1, 27:37 and 3:1 and 15:1 and 3:1 ratios are given in tables 2, 3, 4, and 5. In these tables the ratio of products for repulsion is given as ad/bc and for coupling as bc/ad. This is done because it is more convenient to keep the ratios less than one for all crossover percentages less than 50.

TABLE 3

Constants to facilitate the calculation of linkage intensities and their probable errors when one character pair is determined by two complementary factor differences and the other character pair by a single factor difference — 9:7 and 3:1 ratios.

CROSSOVER VALUE	RATIO OF PRODUCTS		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN PROBABLE ERROR	
	ad/bc REPULSION	*bc/ad* COUPLING	F_2 REPULSION	F_2 COUPLING
.01	.3335	.005185	34.83	.1088
.02	.3340	.01075	17.42	.1520
.03	.3348	.01670	11.62	.1843
.04	.3360	.02306	8.719	.2107
.05	.3375	.02983	6.980	.2336
.06	.3394	.03703	5.822	.2538
.07	.3416	.04467	4.995	.2722
.08	.3441	.05277	4.376	.2891
.09	.3470	.06133	3.895	.3048
.10	.3503	.07038	3.510	.3195
.11	.3539	.07992	3.196	.3335
.12	.3579	.08997	2.935	.3468
.13	.3623	.1005	2.714	.3596
.14	.3671	.1117	2.525	.3719
.15	.3723	.1233	2.362	.3838
.16	.3779	.1356	2.219	.3953
.17	.3840	.1484	2.093	.4066
.18	.3904	.1618	1.982	.4175
.19	.3974	.1759	1.882	.4283
.20	.4048	.1905	1.792	.4390
.21	.4127	.2059	1.711	.4494
.22	.4211	.2219	1.638	.4597
.23	.4300	.2385	1.571	.4700
.24	.4394	.2559	1.510	.4801
.25	.4495	.2740	1.454	.4902
.26	.4601	.2928	1.402	.5003
.27	.4713	.3123	1.354	.5103
.28	.4832	.3327	1.309	.5203
.29	.4958	.3538	1.268	.5303
.30	.5090	.3756	1.229	.5404
.31	.5231	.3983	1.193	.5505
.32	.5378	.4219	1.159	.5606
.33	.5534	.4462	1.127	.5709
.34	.5699	.4715	1.097	.5812
.35	.5873	.4975	1.069	.5916
.36	.6056	.5245	1.042	.6022
.37	.6249	.5524	1.016	.6129
.38	.6453	.5812	.9922	.6237
.39	.6668	.6108	.9692	.6348
.40	.6894	.6415	.9474	.6459
.41	.7134	.6730	.9265	.6573
.42	.7387	.7055	.9066	.6689

TABLE 3 (*continued*)

CROSSOVER VALUE	RATIO OF PRODUCTS		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN PROBABLE ERROR	
	ad/bc REPULSION	*bc/ad* COUPLING	F_2 REPULSION	F_2 COUPLING
.43	.7653	.7390	.8875	.6808
.44	.7935	.7734	.8692	.6930
.45	.8233	.8087	.8516	.7054
.46	.8548	.8451	.8347	.7181
.47	.8880	.8824	.8184	.7312
.48	.9232	.9206	.8026	.7446
.49	.9605	.9598	.7874	.7585
.50	1.0000	1.0000	.7727	.7727
.51	1.0418	1.0412	.7585	.7874
.52	1.0862	1.0831	.7446	.8026
.53	1.1333	1.1261	.7312	.8184
.54	1.1833	1.1699	.7181	.8347
.55	1.2365	1.2147	.7054	.8516
.56	1.2930	1.2602	.6930	.8692
.57	1.3532	1.3066	.6808	.8875
.58	1.4174	1.3538	.6689	.9066
.59	1.4859	1.4018	.6573	.9265
.60	1.5589	1.4505	.6459	.9474

The values of the ratio of products ad/bc and bc/ad corresponding to crossover values of .01, .02, .03, .04, ... , .50 for two 3:1 ratios (table 2) are taken from the paper by FISHER and BALMUKAND (1928) by permission of the JOURNAL OF GENETICS.

The determination of linkage intensities by the product method then simply resolves itself into calculating ad/bc or bc/ad from the four observed F_2 phenotypic classes and finding the crossover percentage by interpolation in the tables appended. (See tables 2, 3, 4 and 5). Since most determinations of linkage intensities are made from two 3:1 ratios, the interval between the values of p in table 2 has been made .005. In tables 3, 4 and 5 the interval is .01. In the linkage calculations from 9:7 and 3:1, 27:37 and 3:1 or 15:1 and 3:1 ratios, where complementary or duplicate factors are concerned in the production of one of the character pairs, a linkage is assumed between but one of the factor pairs responsible for the 9:7, 27:37 or 15:1 ratio and the single factor pair responsible for the 3:1 ratio.

The probable errors for the four types of ratios dealt with in tables 2, 3, 4 and 5 are obtained by dividing the probable error factor corresponding to the calculated crossover value by the square root of the number of individuals ($\sqrt{N}$).

TABLE 4

Constants to facilitate the calculation of linkage intensities and their probable errors when one character pair is determined by three complementary factor differences and the other character pair by a single factor difference — 27:37 and 3:1 ratios.

CROSSOVER VALUE	RATIO OF PRODUCTS		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN PROBABLE ERROR	
	ad/bc REPULSION	*bc/ad* COUPLING	F_2 REPULSION	F_2 COUPLING
.01	.4668	.008939	51.20	.1265
.02	.4672	.01826	25.60	.1780
.03	.4680	.02797	17.07	.2170
.04	.4690	.03808	12.80	.2495
.05	.4703	.04860	10.24	.2779
.06	.4719	.05952	8.537	.3033
.07	.4738	.07086	7.318	.3266
.08	.4760	.08263	6.405	.3482
.09	.4785	.09482	5.694	.3685
.10	.4813	.1075	5.126	.3876
.11	.4844	.1205	4.661	.4059
.12	.4878	.1341	4.274	.4233
.13	.4916	.1480	3.946	.4402
.14	.4957	.1625	3.666	.4564
.15	.5001	.1774	3.422	.4722
.16	.5049	.1928	3.209	.4876
.17	.5100	.2086	3.022	.5027
.18	.5155	.2249	2.855	.5175
.19	.5214	.2418	2.706	.5320
.20	.5277	.2591	2.571	.5464
.21	.5343	.2768	2.450	.5606
.22	.5414	.2951	2.339	.5747
.23	.5489	.3139	2.238	.5887
.24	.5568	.3332	2.146	.6026
.25	.5652	.3530	2.061	.6165
.26	.5740	.3732	1.982	.6304
.27	.5834	.3940	1.909	.6443
.28	.5932	.4153	1.842	.6583
.29	.6036	.4371	1.779	.6723
.30	.6145	.4594	1.720	.6864
.31	.6260	.4821	1.665	.7007
.32	.6381	.5054	1.613	.7151
.33	.6508	.5292	1.564	.7296
.34	.6642	.5534	1.519	.7443
.35	.6782	.5781	1.475	.7592
.36	.6930	.6033	1.435	.7744
.37	.7085	.6290	1.396	.7898
.38	.7248	.6551	1.359	.8054
.39	.7419	.6817	1.324	.8214
.40	.7599	.7087	1.291	.8380

TABLE 4 (*continued*)

CROSSOVER VALUE	RATIO OF PRODUCTS		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN PROBABLE ERROR	
	ad/bc REPULSION	*bc/ad* COUPLING	F_2 REPULSION	F_2 COUPLING
.41	.7788	.7361	1.259	.8544
.42	.7987	.7640	1.228	.8714
.43	.8196	.7922	1.199	.8889
.44	.8416	.8209	1.172	.9068
.45	.8647	.8499	1.145	.9252
.46	.8891	.8793	1.119	.9441
.47	.9147	.9090	1.094	.9636
.48	.9416	.9390	1.071	.9836
.49	.9701	.9694	1.048	1.004
.50	1.0000	1.0000	1.026	1.026
.51	1.0316	1.0309	1.004	1.048
.52	1.0649	1.0620	.9836	1.071
.53	1.1001	1.0933	.9636	1.094
.54	1.1373	1.1248	.9441	1.119
.55	1.1766	1.1565	.9252	1.145
.56	1.2182	1.1882	.9068	1.172
.57	1.2623	1.2201	.8889	1.199
.58	1.3089	1.2520	.8714	1.228
.59	1.3585	1.2840	.8544	1.259
.60	1.4111	1.3160	.8380	1.291

As an example of the use of the product method, assume a cross AABB × aabb would give this ratio in F_2:

AB(a)	Ab(b)	aB(c)	ab(d)	Total(N)
1757	118	119	506	2500

Since the cross is one of coupling, bc/ad = 118 × 119/1757 × 506 = 0.01579. By interpolation in table 2 we find the crossover value to be 0.0998 which would mean 9.98 percent crossing over. From the same table we find by interpolation that the proper factor for the probable error is 0.2151 which, divided by $\sqrt{2500}$ gives a probable error of the above linkage intensity of 0.0043 or 0.43 percent.

If two or three factor pairs had been involved in determining one of the character pairs from a cross made in the coupling phase, we would have calculated bc/ad as before and looked up the crossover percentage from table 3, 4 or 5, depending on whether two or three complementary or duplicate factor pairs were concerned.

TABLE 5

Constants to facilitate the calculation of linkage intensities and their probable errors when one character pair is determined by two duplicate factor differences and the other character pair by a single factor difference — 15:1 and 3:1 ratios.

CROSSOVER VALUE	RATIO OF PRODUCTS		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN PROBABLE ERROR	
	ad/bc REPULSION	*bc/ad* COUPLING	F_3 REPULSION	F_3 COUPLING
.01	.0002750	.005118	1.349	.1895
.02	.001101	.01048	1.349	.2664
.03	.002478	.01609	1.348	.3243
.04	.004409	.02197	1.348	.3723
.05	.006898	.02811	1.347	.4139
.06	.009948	.03455	1.346	.4509
.07	.01356	.04127	1.345	.4846
.08	.01775	.04831	1.343	.5154
.09	.02252	.05567	1.342	.5440
.10	.02787	.06336	1.340	.5708
.11	.03382	.07140	1.338	.5959
.12	.04038	.07981	1.336	.6197
.13	.04755	.08860	1.334	.6423
.14	.05535	.09778	1.331	.6638
.15	.06379	.1074	1.329	.6843
.16	.07288	.1174	1.326	.7041
.17	.08265	.1279	1.323	.7230
.18	.09311	.1389	1.320	.7412
.19	.1043	.1504	1.317	.7588
.20	.1162	.1624	1.313	.7758
.21	.1288	.1749	1.309	.7923
.22	.1422	.1881	1.306	.8083
.23	.1564	.2018	1.302	.8238
.24	.1714	.2162	1.297	.8389
.25	.1873	.2312	1.293	.8535
.26	.2041	.2470	1.289	.8678
.27	.2217	.2635	1.284	.8817
.28	.2403	.2808	1.279	.8953
.29	.2599	.2989	1.274	.9085
.30	.2805	.3180	1.269	.9215
.31	.3022	.3379	1.264	.9342
.32	.3250	.3588	1.258	.9465
.33	.3489	.3808	1.252	.9586
.34	.3740	.4039	1.247	.9705
.35	.4004	.4281	1.241	.9821
.36	.4282	.4536	1.235	.9935
.37	.4573	.4804	1.228	1.005
.38	.4878	.5086	1.222	1.016
.39	.5199	.5383	1.215	1.026
.40	.5536	.5696	1.208	1.037

TABLE 5 (*continued*)

CROSSOVER VALUE	RATIO OF PRODUCTS		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN PROBABLE ERROR	
	ad/bc REPULSION	*bc/ad* COUPLING	F_2 REPULSION	F_2 COUPLING
.41	.5889	.6027	1.201	1.047
.42	.6261	.6375	1.194	1.057
.43	.6651	.6743	1.187	1.067
.44	.7060	.7132	1.179	1.077
.45	.7491	.7543	1.172	1.086
.46	.7943	.7979	1.164	1.096
.47	.8419	.8440	1.156	1.105
.48	.8919	.8929	1.148	1.114
.49	.9446	.9448	1.140	1.123
.50	1.0000	1.0000	1.131	1.131
.51	1.0584	1.0587	1.123	1.140
.52	1.1200	1.1212	1.114	1.148
.53	1.1849	1.1878	1.105	1.156
.54	1.2534	1.2590	1.096	1.164
.55	1.3257	1.3350	1.086	1.172
.56	1.4022	1.4164	1.077	1.179
.57	1.4830	1.5037	1.067	1.187
.58	1.5686	1.5973	1.057	1.194
.59	1.6593	1.6980	1.047	1.201
.60	1.7555	1.8065	1.037	1.208

SUMMARY

1. The development of probable error formulae for various methods of calculating linkage intensities from F_2 data has provided a means of determining the relative efficiency of these methods.

2. The product method (equivalent to the coefficient of association method) seems to be the best general method available. The ratio of products ad/bc or bc/ad is calculated from the observed data and the linkage intensity obtained by interpolation in the tables appended.

3. F_2 coupling data are but slightly less reliable than backcross data while F_2 repulsion data are less reliable, the relative reliability depending on the closeness of the linkage. In linkage studies where backcrossing is difficult or time consuming, linkage intensities may be accurately determined from F_2 data by growing slightly larger populations. This is particularly true of the coupling phase.

4. Linkage intensities calculated from F_2 coupling data are more reliable as measured by the probable error, than from F_2 repulsion data, the relative reliability depending on the closeness of the linkage. For close linkage, coupling data are much more reliable than repulsion data.

5. Linkages between 2 factor pairs may be determined quite accurately from 9:7 and 3:1 or 27:37 and 3:1 ratios if the cross is made in the coupling phase. In the repulsion phase much larger numbers are needed to obtain the same reliability. When duplicate factors are involved (15:1 and 3:1 or 63:1 and 3:1 ratios) much larger populations are needed to obtain the same reliability as would be secured from two 3:1 ratios.

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FRONTISPIECE

Professor Doctor CARL WILHELM VON NÄGELI, botanical investigator, teacher and philosopher, was born at Kilchberg, near Zürich, Switzerland, March 26, 1817, and died in München, Germany, May 10, 1891. He was one of the most versatile and most eminent botanists of his day. To geneticists he will doubtless always be best known as the distinguished botanist who, fully informed of MENDEL'S studies on the garden pea, failed to recognize their fundamental significance, owing, one may think, to NÄGELI'S own intense and long-continued interest in Hieracium, which must have greatly influenced his judgment as to which of MENDEL'S studies were of highest importance.

NÄGELI'S publications on hybridization, mostly published about 1865, were mainly discussions of phenomena recorded by previous hybridologists, KOELREUTER and GAERTNER, while his big work on "Mechanisch-physiologische Theorie der Abstammungslehre," published in 1884, is mainly philosophical rather than experimental, and closely resembles WEISMANN'S work in the clear-cut conception of the idioplasm" as the inheriting substance in contrast to the stereoplasm." He did not agree with WEISMANN, however, in his attitude toward the inheritance of acquired characters.

By those who knew him best the portrait here reproduced was recognized as the most excellent likeness of NÄGELI in existence. The Editorial Board is deeply indebted to Doctor C. CORRENS, who was a student of NÄGELI and who is his grand-nephew by marriage, for generous assistance in securing this portrait, signature and biographical data.

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